

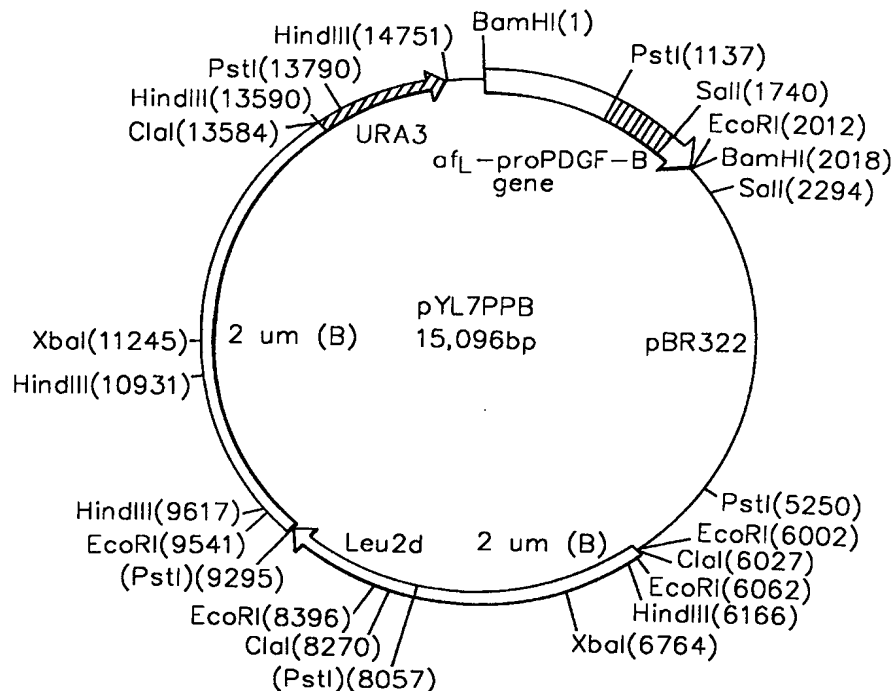


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(54) Title: METHOD FOR EXPRESSION OF HETEROLOGOUS PROTEINS IN YEAST**(57) Abstract**

Compositions and methods for expression of heterologous mammalian proteins and their secretion in the biologically active mature form using a yeast host cell as the expression system are provided. Compositions of the invention are nucleotide sequences encoding a signal peptide sequence for a yeast secreted protein, an optional leader peptide sequence for a yeast secreted protein, a native propeptide leader sequence for a mature protein of interest, and a sequence for the mature protein of interest, all operably linked to a yeast promoter. Each of these elements is associated with a processing site recognized in vivo by a yeast proteolytic enzyme. Any or all of these processing sites may be a preferred processing site that has been modified or synthetically derived for more efficient cleavage in vivo. The compositions are useful in methods for expression of heterologous mammalian proteins and their secretion in the biologically active mature form. Particularly, vectors comprising these nucleotide coding sequences can be used to transform a yeast host cell, which can then be cultured and screened for secretion of the biologically active mature protein of interest.



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METHOD FOR EXPRESSION OF HETEROLOGOUS PROTEINS IN YEAST

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FIELD OF THE INVENTION

The present invention relates to the production of recombinant proteins using yeast host cells as the expression system. More particularly, it relates to compositions and methods for expression of heterologous proteins and their secretion as the biologically active mature proteins.

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BACKGROUND OF THE INVENTION

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Yeast host expression systems have been used to express and secrete proteins foreign to yeast. Numerous approaches have been developed in terms of the degree of expression and the yield of biologically active mature proteins.

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Such approaches have involved modifications to the various molecular components that are involved in expression and secretion of proteins in yeast. These components include the translation and termination regulatory regions for gene expression; signal peptide and secretion leader peptide sequences, which direct the precursor form of the heterologous protein through the yeast secretory pathway; and processing sites, which cleave leader peptide sequences from the polypeptide sequence of the protein of interest.

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Expression of the protein of interest can be enhanced with use of yeast-recognized regulatory regions. Increased yield of the heterologous protein of interest is commonly achieved with the use of yeast-derived signal and secretion leader peptide sequences. The use of native signal-leader peptide sequences is believed to improve direction of the protein of interest through the secretory pathway of the yeast host.

30

Previous work has demonstrated that full-length yeast α -factor signal-leader sequences can be used to drive expression and processing of heterologous proteins in yeast host cells. Substantial improvements in efficiency of expression can be accomplished with the use of truncated α -factor leader sequences, particularly for heterologous proteins that are poorly expressed by the full-length sequence or whose expression is nonresponsive to the full-length sequence.

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Methods for expression of heterologous proteins and their secretion in a biologically active mature form using a yeast host cell as the expression system are needed.

Compositions and methods for expression of heterologous proteins, more particularly heterologous mammalian proteins, and their secretion in a biologically active mature form using a yeast host cell as the expression system are provided. Compositions of the invention are nucleotide sequences encoding a signal sequence for a yeast secreted protein, a native propeptide leader sequence for a mature protein of interest, and a peptide sequence for the mature protein of interest. Each of these elements is associated with a processing site recognized *in vivo* by a yeast proteolytic enzyme. Any or all of these processing sites may be a preferred processing site that has been modified or synthetically derived for more efficient cleavage *in vivo*. In turn, all of these elements are operably linked to a yeast promoter and optionally other regulatory sequences.

The nucleotide coding sequences of these compositions may additionally comprise a leader peptide sequence for a yeast secreted protein. When present, this element, which is also associated with a processing site recognized in vivo by a yeast proteolytic enzyme, is positioned 3' to the yeast signal sequence and 5' to the sequence for the mature protein of interest. Thus cleavage by a yeast proteolytic enzyme removes the yeast leader sequence from the hybrid precursor molecule comprising the sequence for the mature protein of interest.

These compositions are useful in methods for expression of heterologous mammalian proteins and their secretion in the biologically active mature form. Particularly, vectors comprising these nucleotide coding sequences can be used to

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5 transform a yeast host cell, which can then be cultured and screened for secretion of the biologically active mature protein of interest.

The method of the present invention is particularly useful in production of mammalian proteins whose assumption of a native conformation is facilitated by the presence of a native propeptide sequence in the precursor polypeptide.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a map of plasmid pAB24.

Figure 2 is a map of the rhPDGF-B expression cassette in pAGL7PB and
15 pYAGL7PB.

Figure 3 is a map of rhPDGF-B expression plasmid pYAGL7PB.

Figure 4 is a map of the rhPDGF-B expression cassette in pL7PPB and pYL7PPB.

Figure 5 shows the final steps in the construction of the rhPDGF-B expression
cassette in pL7PPB.

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Figure 6 is a map of rhPDGF-B expression plasmid pYL7PPB.

DETAILED DESCRIPTION OF THE INVENTION

25 The present invention provides compositions and methods for expression of heterologous proteins of interest, more particularly heterologous mammalian proteins, and their secretion in a biologically active mature form using a yeast host cell as the expression system. By "biologically active mature form" is intended a protein whose conformational form is similar to the native conformation such that its biological activity is substantially the same as the biological activity of the native protein.

30 Compositions of the present invention are nucleotide sequences encoding hybrid precursor polypeptides that each comprise the polypeptide sequence for a mature heterologous protein of interest. Expression vectors comprising these nucleotide sequences, all under the operational control of a yeast promoter region and a yeast terminator region, are also provided. Methods of the invention comprise stably
35 transforming a yeast host cell with said vectors, where expression of the nucleotide sequence encoding the hybrid precursor polypeptide leads to secretion of the mature heterologous protein of interest in a biologically active form.

By "heterologous protein of interest" is intended a protein that is not expressed by the yeast host cell in nature. Preferably the heterologous protein will be a

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5 mammalian protein, including substantially homologous and functionally equivalent
variants thereof. By "variant" is intended a polypeptide derived from the native
polypeptide by deletion (so-called truncation) or addition of one or more amino acids
to the N-terminal and/or C-terminal end of the native protein; deletion or addition of
one or more amino acids at one or more sites in the native polypeptide; or substitution
10 of one or more amino acids at one or more sites in the native polypeptide. Such
variants may result from, for example, genetic polymorphism or from human
manipulation. Methods for such manipulations are generally known in the art.

For example, amino acid sequence variants of the polypeptide can be prepared
by mutations in the cloned DNA sequence encoding the native polypeptide of interest.
15 Methods for mutagenesis and nucleotide sequence alterations are well known in the
art. See, for example, Walker and Gaastra, eds. (1983) *Techniques in Molecular
Biology* (MacMillan Publishing Company, New York); Kunkel (1985) *Proc. Natl.
Acad. Sci. USA* 82:488-492; Kunkel *et al.* (1987) *Methods Enzymol.* 154:367-382;
Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring
20 Harbor, New York); U.S. Patent No. 4,873,192; and the references cited therein;
herein incorporated by reference. Guidance as to appropriate amino acid substitutions
that do not affect biological activity of the protein of interest may be found in the
model of Dayhoff *et al.* (1978) in *Atlas of Protein Sequence and Structure* (Natl.
Biomed. Res. Found., Washington, D.C.), herein incorporated by reference.

25 Conservative substitutions, such as exchanging one amino acid with another having
similar properties, may be preferred. Examples of conservative substitutions include,
but are not limited to, Gly \leftrightarrow Ala, Val \leftrightarrow Ile \leftrightarrow Leu, Asp \leftrightarrow Glu, Lys \leftrightarrow Arg, Asn \leftrightarrow Gln,
and Phe \leftrightarrow Trp \leftrightarrow Tyr.

In constructing variants of the protein of interest, modifications will be made
30 such that variants continue to possess the desired activity. Obviously, any mutations
made in the DNA encoding the variant protein must not place the sequence out of
reading frame and preferably will not create complementary regions that could
produce secondary mRNA structure. See EP Patent Application Publication No.
75,444.

35 Thus proteins of the invention include the naturally occurring forms as well as
variants thereof. These variants will be substantially homologous and functionally
equivalent to the native protein. A variant of a native protein is "substantially

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homologous" to the native protein when at least about 80%, more preferably at least about 90%, and most preferably at least about 95% of its amino acid sequence is identical to the amino acid sequence of the native protein. A variant may differ by as few as 1, 2, 3, or 4 amino acids. By "functionally equivalent" is intended that the sequence of the variant defines a chain that produces a protein having substantially the same biological activity as the native protein of interest. Such functionally equivalent variants that comprise substantial sequence variations are also encompassed by the invention. Thus a functionally equivalent variant of the native protein will have a sufficient biological activity to be therapeutically useful. By "therapeutically useful" is intended effective in achieving a therapeutic goal, as, for example, healing a wound.

Methods are available in the art for determining functional equivalence. Biological activity can be measured using assays specifically designed for measuring activity of the native protein, including assays described in the present invention. Additionally, antibodies raised against the biologically active native protein can be tested for their ability to bind to the functionally equivalent variant, where effective binding is indicative of a protein having a conformation similar to that of the native protein.

The nucleotide sequences encoding the mature heterologous proteins of interest can be sequences cloned from non-yeast organisms, or they may be synthetically derived sequences, usually prepared using yeast-preferred codons. Examples of heterologous proteins suitable for the invention include, but are not limited to transforming growth factor (TGF-alpha and TGF-beta), somatostatin (as in SRIF 1), parathyroid hormone, and more particularly platelet-derived growth factor (PDGF) and insulin growth factor (IGF), all of which have a native prosequence as part of the precursor protein.

Thus compositions of the present invention are nucleotide sequences encoding hybrid precursor polypeptides that each comprise the polypeptide sequence for a mature heterologous protein of interest or any substantially homologous and functionally equivalent variants thereof. More particularly, nucleotide sequences of the present invention encode in the 5' to 3' direction a hybrid precursor polypeptide comprising the following primary elements:



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wherein:

SP comprises a signal peptide sequence for a yeast secreted protein;

PS comprises a processing site cleaved in vivo by a yeast proteolytic enzyme;

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LP comprises a leader peptide sequence for a yeast secreted protein;

NPRO_{MHP} comprises a native N-terminal propeptide sequence of a mature heterologous protein of interest;

MHP comprises a peptide sequence for said mature heterologous mammalian protein of interest;

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CPRO_{MHP} comprises a native C-terminal propeptide sequence of said mature heterologous mammalian protein of interest; and

n-1, n-2, n-3, and n-4 independently = 0 or 1;

wherein said processing sites allow for proteolytic processing of said precursor polypeptide to said mature protein in vivo by a yeast host cell, and wherein at least n-3 or n-4 = 1.

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As is the case for the heterologous protein of interest, each of the other elements present in the hybrid precursor polypeptide can be a known naturally occurring polypeptide sequence or can be synthetically derived, including any variants thereof that do not adversely affect the function of the element as described herein. By "adversely affect" is intended inclusion of the variant form of the element results in decreased yield of the secreted mature heterologous protein of interest relative to the hybrid precursor polypeptide comprising the native form of the element.

25

In constructing the nucleotide sequence encoding the hybrid precursor polypeptide, it is within skill in the art to employ adapters or linkers to join the nucleotide fragments encoding the various elements of the precursor polypeptide. See, for example, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, New York). Thus, the hybrid precursor polypeptide may comprise additional elements positioned 5' or 3' to any of the primary elements listed above, including the yeast leader peptide sequence and its associated yeast-recognized processing site when present.

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For purposes of the present invention, SP is a presequence that is an N-terminal sequence for the precursor polypeptide of the mature form of a yeast secreted

5 protein. When the nucleotide sequence encoding the hybrid precursor polypeptide is
expressed in a transformed yeast host cell, the signal peptide sequence functions to
direct the hybrid precursor polypeptide comprising the mature heterologous protein of
interest into the endoplasmic reticulum (ER). Movement into the lumen of the ER
represents the initial step into the secretory pathway of the yeast host cell. Although
10 the signal peptide of the invention can be heterologous to the yeast host cell, more
preferably the signal peptide will be native to the host cell.

The signal peptide sequence of the invention may be a known naturally
occurring signal sequence or any variant thereof as described above that does not
adversely affect the function of the signal peptide. Examples of signal peptides
15 appropriate for the present invention include, but are not limited to, the signal peptide
sequences for α -factor (see, for example, U.S. Patent No. 5,602,034; Brake *et al.*
(1984) *Proc. Natl. Acad. Sci. USA* 81:4642-4646); invertase (WO 84/01153); PHO5
(DK 3614/83); YAP3 (yeast aspartic protease 3; PCT Publication No. 95/02059); and
BAR1 (PCT Publication No. 87/02670). Alternatively, the signal peptide sequence
20 may be determined from genomic or cDNA libraries using hybridization probe
techniques available in the art (see Sambrook *et al.* (1989) *Molecular Cloning: A
Laboratory Manual* (Cold Spring Harbor, New York), or even synthetically derived
(see, for example, WO 92/11378).

During entry into the ER, the signal peptide is cleaved off the precursor
25 polypeptide at a processing site. The processing site can comprise any peptide
sequence that is recognized in vivo by a yeast proteolytic enzyme. This processing
site may be the naturally occurring processing site for the signal peptide. More
preferably, the naturally occurring processing site will be modified, or the processing
site will be synthetically derived, so as to be a preferred processing site. By "preferred
30 processing site" is intended a processing site that is cleaved in vivo by a yeast
proteolytic enzyme more efficiently than is the naturally occurring site. Examples of
preferred processing sites include, but are not limited to, dibasic peptides, particularly
any combination of the two basic residues Lys and Arg, that is Lys-Lys, Lys-Arg,
Arg-Lys, or Arg-Arg, most preferably Lys-Arg. These sites are cleaved by the
35 endopeptidase encoded by the KEX2 gene of *Saccharomyces cerevisiae* (see Fuller *et al.*
Microbiology 1986:273-278) or the equivalent protease of other yeast species (see
Julius *et al.* (1983) *Cell* 32:839-852). In the event that the KEX2 endopeptidase would

5 cleave a site within the peptide sequence for the mature heterologous protein of interest, other preferred processing sites could be utilized such that the peptide sequence of interest remains intact (see, for example, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, New York).

10 A functional signal peptide sequence is essential to bring about extracellular secretion of a heterologous protein from a yeast cell. Additionally, the hybrid precursor polypeptide may comprise a secretion leader peptide sequence of a yeast secreted protein to further facilitate this secretion process. When present, the leader peptide sequence is generally positioned immediately 3' to the signal peptide sequence processing site. By "secretion leader peptide sequence" (LP) is intended a
15 peptide that directs movement of a precursor polypeptide, which for the purposes of this invention is the hybrid precursor polypeptide comprising the mature heterologous protein to be secreted, from the ER to the Golgi apparatus and from there to a secretory vesicle for secretion across the cell membrane into the cell wall area and/or the growth medium. The leader peptide sequence may be native or heterologous to the
20 yeast host cell but more preferably is native to the host cell.

The leader peptide sequence of the present invention may be a naturally occurring sequence for the same yeast secreted protein that served as the source of the signal peptide sequence, a naturally occurring sequence for a different yeast secreted protein, or a synthetic sequence (see, for example, WO 92/11378), or any variants
25 thereof that do not adversely affect the function of the leader peptide.

For purposes of the invention, the leader peptide sequence when present is preferably derived from the same yeast secreted protein that served as the source of the signal peptide sequence, more preferably an α -factor protein. A number of genes encoding precursor α -factor proteins have been cloned and their combined signal-
30 leader peptide sequences identified. See, for example, Singh *et al.* (1983) *Nucleic Acids Res.* 11:4049-4063; Kurjan *et al.*, U.S. Patent No. 4,546,082; U.S. Patent No. 5,010,182; herein incorporated by reference. Alpha-factor signal-leader peptide sequences have been used to express heterologous proteins in yeast. See, for example, Elliott *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:7080-7084; Bitter *et al.* (1984)
35 *Proc. Natl. Acad. Sci.* 81:5330-5334; Smith *et al.* (1985) *Science* 229:1219-1229; and U.S. Patent Nos. 4,849,407 and 5,219,759; herein incorporated by reference.

5 Alpha-factor, an oligopeptide mating pheromone approximately 13 residues in length, is produced from a larger precursor polypeptide of between about 100 and 200 residues in length, more typically about 120-160 residues. This precursor polypeptide comprises the signal sequence, which is about 19-23 (more typically 20-22 residues), the leader sequence, which is about 60 residues, and typically 2-6 tandem repeats of
10 the mature pheromone sequence. Although the signal peptide sequence and full-length α -factor leader peptide sequence can be used, more preferably for this invention a truncated α -factor leader peptide sequence will be used with the signal peptide when both elements are present in the hybrid precursor molecule.

 By "truncated" α -factor leader peptide sequence is intended a portion of the
15 full-length α -factor leader peptide sequence that is about 20 to about 60 amino acid residues, preferably about 25 to about 50 residues, more preferably about 30 to about 40 residues in length. Methods for using truncated α -factor leader sequences to direct secretion of heterologous proteins in yeast are known in the art. See particularly U.S. Patent No. 5,602,034. When the hybrid precursor polypeptide sequence comprises a
20 truncated α -factor leader peptide, deletions to the full-length leader will preferably be from the C-terminal end and will be done in such a way as to retain at least one glycosylation site (-Asn-Y-Thr/Ser-, where Y is any amino acid residue) in the truncated peptide sequence. This glycosylation site, whose modification is within skill in the art, is retained to facilitate secretion (see particularly WO 89/02463).

25 When the hybrid precursor polypeptide sequence of the present invention comprises a leader peptide sequence, such as the α -factor leader sequence, there will be a processing site immediately adjacent to the 3' end of the leader peptide sequence. This processing site enables a proteolytic enzyme native to the yeast host cell to cleave the yeast secretion leader peptide sequence from the 5' end of the native N-
30 terminal propeptide sequence of the mature heterologous protein of interest, when present, or from the 5' end of the peptide sequence for the mature heterologous protein of interest. The processing site can comprise any peptide sequence that is recognized in vivo by a yeast proteolytic enzyme such that the mature heterologous protein of interest can be processed correctly. The peptide sequence for this
35 processing site may be a naturally occurring peptide sequence for the native processing site of the leader peptide sequence. More preferably, the naturally

5 occurring processing site will be modified, or the processing site will be synthetically derived, so as to be a preferred processing site as described above.

In the present invention, the nucleotide sequence encoding the hybrid precursor polypeptide comprises a native propeptide sequence (PRO_{MHP}) for the mature heterologous protein of interest. By "native propeptide sequence" or "native prosequence" is intended that portion of an intermediate precursor polypeptide (which is called a "pro-protein") for a mature secreted protein that remains attached to the N-terminal and/or C-terminal end of the mature protein sequence following cleavage of the native signal peptide sequence (or presequence) from the initial precursor polypeptide (or "prepro-protein"). The residues of the propeptide sequence are not
10 contained in the mature secreted protein. Rather, such extra residues are removed at processing sites by proteolytic enzymes near the end of the secretory pathway, in the trans-Golgi network (Griffiths and Simons (1986) *Science* 234:438-443) and secretory granules (Orci *et al.* (1986) *J. Cell Biol.* 103:2273-2281).
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The present invention provides for the presence of propeptide sequences that naturally occur at the N-terminal and/or C-terminal end of the native pro-protein precursor form of the mature heterologous protein of interest. Thus, a propeptide sequence may be positioned between the 3' end of the signal peptide sequence processing site, or the 3' end of the yeast-recognized processing site adjacent to the leader peptide sequence if present, and the 5' end of the peptide sequence for the mature heterologous protein of interest (an N-terminal propeptide sequence, PRO_{MHP}) or immediately adjacent to the 3' end of the peptide sequence for the mature heterologous protein of interest (a C-terminal propeptide sequence, CPRO_{MHP}), depending on its orientation within the native pro-protein. The invention also provides for inclusion of both an N-terminal and a C-terminal propeptide sequence flanking the peptide sequence for the mature heterologous protein of interest when both propeptide sequences exist in the native pro-protein. Where both an N-terminal and a C-terminal propeptide sequence exists in the native pro-protein, preference for inclusion of both propeptide sequences in the hybrid precursor polypeptide will be experimentally determined.
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35 Methods are available in the art for determining the naturally occurring processing sites for the native signal peptide and propeptide sequences of a prepro-protein (see, for example, von Heijne (1983) *Eur. J. Biochem.* 133:17-21, (1984) *J. Mol. Biol.* 173: 243-251, (1986) *J. Mol. Biol.* 184:99-105, and (1986) *Nucleic Acids*

5 *Res.* 14:4683-4690) such that the native N-terminal and/or C-terminal propeptide sequence can be determined for use in the invention.

 Immediately 3' to the native N-terminal propeptide sequence (when present) or immediately 5' to the C-terminal propeptide sequence (when present) is a processing site that is recognized in vivo by a yeast proteolytic enzyme. This
 10 processing site allows for cleavage of the propeptide sequence from the peptide sequence for the mature heterologous protein of interest (MHP). It is recognized that this processing site may be the naturally occurring processing site for the propeptide sequence if the naturally occurring site is recognized in vivo by a proteolytic enzyme of the yeast host cell. More preferably, the naturally occurring processing site will be
 15 modified, or the processing site will be synthetically derived, so as to be a preferred processing site. Examples of preferred processing sites include, but are not limited to, those discussed above for the other processing. Preferably all of these processing sites will be similar such that the same yeast proteolytic enzyme brings about cleavage of the signal and leader peptide sequences and the native propeptide sequence(s).

20 In accordance with the invention as stated above, the yeast signal peptide and secretion leader peptide sequences, as well as the native propeptide sequences, represent those parts of the hybrid precursor polypeptide of the invention that can direct the sequence for the mature heterologous protein of interest through the secretory pathway of a yeast host cell.

25 In one preferred embodiment of the present invention, the nucleotide sequence of the hybrid precursor polypeptide comprises in the 5' to 3' direction:



30 wherein:

 AFSP comprises an α -factor signal peptide sequence and a processing site;
 tAFLP comprises a truncated α -factor secretion leader peptide sequence;
 PS_L comprises a preferred processing site for the leader peptide sequence;
 NPRO_{PDGF} comprises the peptide sequence for a native N-terminal propeptide of
 35 a mature platelet-derived growth factor (PDGF);
 PS_{NPRO} comprises a preferred processing site for the N-terminal propeptide sequence; and
 M_{PDGF} comprises the sequence for said mature PDGF.

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Preferably the α -factor signal peptide and truncated α -factor secretion leader peptide sequences are derived from the *Mata α* gene of *S. cerevisiae* as outlined in the examples. The preferred truncated α -factor leader peptide sequence will include the N-terminal portion of the full-length leader sequence; that is, the leader sequence will start with the first amino acid residue of the full-length sequence and run the length of about 20 to about 60 amino acid residues, preferably about 25 to about 50 residues, more preferably about 30 to about 40 residues. In one embodiment, a leader of about 35 residues is used.

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The mature protein of this preferred embodiment is human platelet-derived growth factor (PDGF). PDGF, the primary mitogen in serum for mesenchymal-derived cells, is stored in platelet alpha-granules. Injury to blood vessels activates the release of PDGF from these granules in the vicinity of the injured vessels. This mitogen acts as a potent chemoattractant for fibroblasts and smooth muscle cells, as well as monocytes and neutrophils. The mitogenic activity of the localized PDGF results in proliferation of these cells at the site of injury, contributing to the process of wound repair.

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Purified native platelet-derived growth factor (PDGF), a glycoprotein of about 30,000 daltons, is composed of two disulfide-linked polypeptide chains. Two forms of these chains, designated A and B, have been identified. The native protein occurs as the homodimer AA or BB or the heterodimer AB, or a mixture thereof. A partial amino acid sequence for the PDGF-A chain has been identified (Johnsson *et al.* (1984) *EMBO J.* 3:921-928) and cDNAs encoding two forms of PDGF A-chain precursors have been described (U.S. Patent No. 5,219,759). The A-chain is derived by proteolytic processing of a 211 amino acid precursor polypeptide. The cDNA encoding the PDGF-B chain has also been described (*Nature* (1985) 316:748-750). The B-chain is derived by proteolytic processing of a 241 amino acid precursor.

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The mature PDGF protein of the present invention will be the biologically active dimeric form, including the homodimers PDGF-AA and PDGF-BB or the heterodimer PDGF-AB, and any substantially homologous and functionally equivalent variants thereof as defined above. For example, the native amino acid sequence for the A-chain or the B-chain may be truncated at either the N-terminal or C-terminal end. Thus removal of up to 15 or up to 10 amino acids from the N-

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5 terminal or C-terminal end, respectively, of the B-chain does not affect biological activity of the variant. Additionally, amino-acid substitutions may be made. For example, an amino acid such as serine may be substituted for any of the cysteine residues at positions 43, 52, 53, and 97 of the native human B-chain and at
10 corresponding positions in the native A-chain to obtain substantially homologous and functionally equivalent variants of the native chain. Variants of the A-chain are known based on cloned DNA sequences, such as, for example, variants having an additional 6 or 19 amino acids at the C-terminal end. See, for example, Tong *et al.* (1987) *Nature* 328:619-621; Betsholtz *et al.* (1986) *Nature* 320:695-699. One PDGF B-chain variant may be the corresponding substantially homologous portion of the
15 amino-acid sequence encoded by the v-sis gene of simian sarcoma virus. The homologous region of the product of this gene, p28^{sis}, begins at amino acid 67 and continues to amino acid 175, and differs from the human B-chain by only 4 amino acid residues (see, for example, European Patent Application No. 0 487 116 A1). Functionally equivalent variants can be determined with assays for biological activity
20 as described in the examples.

The nucleotide sequence encoding the mature PDGF protein of the present invention may be genomic, cDNA, or synthetic DNA. The genes encoding the native forms of PDGF have been sequenced, and several variants are well known in the art. Expression of PDGF homodimers and heterodimers is described in, for example, U.S.
25 Patent Nos. 4,766,073; 4,769,328; 4,801,542; 4,845,075; 4,849,407; 5,045,633; 5,128,321; and 5,187,263; herein incorporated by reference. Based on the known amino acid sequences for the A- and B-chain polypeptides, synthetic nucleotide sequences encoding PDGF A-chain and B-chain polypeptides may be made *in vitro* using methods available in the art. See particularly Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, New York). Where the mature
30 protein of interest is the heterodimer PDGF-AB, the nucleotide sequences encoding the hybrid precursor polypeptides comprising the A- and B-chain polypeptides may be assembled as part of one expression cassette or assembled into separate expression cassettes for cotransformation of a yeast host cell.

35 In this preferred embodiment comprising mature IGF-I, the C-terminal end of the truncated α -factor secretion leader peptide sequence and of the native N-terminal propeptide sequence will terminate in a preferred processing site, preferably a dibasic

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5 processing site that is specific for the KEX2 endopeptidase of *S. cerevisiae*. The dipeptides can be any combination of the basic residues Lys and Arg, more preferably a Lys-Arg dipeptide.

The native prepro-PDGF-B additionally comprises a 51 amino acid C-terminal propeptide. In another preferred embodiment, the nucleotide sequence encoding the
10 hybrid precursor polypeptide comprises in the 5' to 3' direction the following modified sequence:

5'-AFSP-tAFLP-PS_L-NPRO_{PDGF}-PS_{NPRO}-M_{PDGF}-PS_{CPRO}-CPRO_{PDGF}-3'

15 wherein:

CPRO_{PDGF} comprises a C-terminal propeptide sequence for said PDGF mature heterologous protein of interest; and
20 PS_{CPRO} comprises a preferred processing site for the C-terminal propeptide sequence.

Preferably the preferred processing site for the C-terminal propeptide sequence is similar to that of the leader peptide sequence and the N-terminal propeptide sequence,
25 such that the same yeast proteolytic enzyme brings about cleavage of the α -factor leader peptide sequence and the sequences for both of the native propeptides. Inclusion of these two additional components is experimentally determined.

In another preferred embodiment of the invention, the nucleotide sequence of the hybrid precursor polypeptide comprises in the 5' to 3' direction:

30 5'-AFSP-AFLP-PS_L-M_{IGF}-PS_{CPRO}-CPRO_{IGF}-3'

wherein:

AFSP comprises an α -factor signal peptide sequence and a processing site;
35 AFLP comprises an α -factor secretion leader peptide sequence
PS_L comprises a preferred processing site for the leader peptide sequence;
M_{IGF} comprises the peptide sequence for a mature insulin-like growth factor (IGF);
PS_{CPRO} comprises a preferred processing site for the C-terminal propeptide
40 sequence; and

5 CPRO^{IGF} comprises the peptide sequence for a native C-terminal propeptide of
said mature IGF.

10 Preferably the α -factor signal peptide and α -factor secretion leader peptide
sequences are derived from the Mata α gene of *S. cerevisiae* as outlined for the
preferred embodiment for PDGF.

15 The mature protein of this preferred embodiment is insulin-like growth factor
(IGF), more particularly IGF-I. Insulin-like growth factor (IGF-I) belongs to a family
of polypeptides known as somatomedins. IGF-I stimulates growth and division of a
variety of cell types, particularly during development. See, for example, European
Patent Application Nos. 560,723 A and 436,469 B. Thus, processes such as skeletal
growth and cell replication are affected by IGF-I levels.

20 IGF-I is structurally and functionally similar to, but antigenically
distinct from, insulin. In this regard, IGF-I is a single-chain polypeptide with three
intrachain disulfide bridges and four domains known as the A, B, C, and D domains,
respectively. The A and B domains are connected by the C domain, and are
homologous to the corresponding domains of proinsulin. The D domain, a C-terminal
prosequence, is present in IGF-I but is absent from proinsulin. IGF-I has 70 amino
acid residues and a molecular mass of approximately 7.5 kDa. See Rinderknecht
(1978) *J. Biol. Chem.* 253:2769 and *FEBS Lett.* 89:283. For a review of IGF, see
25 Humbel (1990) *Eur. J. Biochem.* 190:445-462.

30 The mature IGF protein of the present invention will be the biologically active
form and any substantially homologous and functionally equivalent variants thereof as
defined above. Functionally equivalent variants can be determined with assays for
biological activity, including the assay, as described in the examples. Representative
assays include known radioreceptor assays using placental membranes (see, for
example, U.S. Patent No. 5,324,639; Hall *et al.* (1974) *J. Clin. Endocrinol. and
Metab.* 39:973-976; and Marshall *et al.* (1974) *J. Clin. Endocrinol. and Metab.*
39:283-292), a bioassay that measures the ability of the molecule to enhance
incorporation of tritiated thymidine, in a dose-dependent manner, into the DNA of
35 BALB/c 3T3 fibroblasts (see, for example, Tamura *et al.* (1989) *J. Biol. Chem.*
262:5616-5621), and the like; herein incorporated by reference.

5 The art provides substantial guidance regarding the preparation and use of IGF-I variants. For example, fragment of IGF-I will generally include at least about 10 contiguous amino acid residues of the full-length molecule, preferably about 15-25 contiguous amino acid residues of the full-length molecule, and most preferably about 20-50 or more contiguous amino acid residues of full-length IGF-I. The term "IGF-I analog" also captures peptides having one or more peptide mimics ("peptoids"), such as those described in International Publication No. WO 91/04282. Several IGF-I analogs and fragments are known in the art and include those described in, for example, *Proc. Natl. Acad. Sci. USA* (1986) 83:4904-4907; *Biochem. Biophys. Res. Commun.* (1987) 149:398-404; *J. Biol. Chem.* (1988) 263:6233-6239; *Biochem. Biophys. Res. Commun.* (1989) 165:766-771; Forsberg *et al.* (1990) *Biochem. J.* 271:357-363; U.S. Patent Nos. 4,876,242 and 5,077,276; International Publication No. WO 87/01038 and WO 89/05822; herein incorporated by reference.

15 Representative analogs include one with a deletion of Glu-3 of the mature molecule, analogs with up to five amino acids truncated from the N-terminus, an analog with a truncation of the first three N-terminal amino acids and an analog including the first 17 amino acids of the B chain of human insulin in place of the first 16 amino acids of human IGF-I.

20 The nucleotide sequence encoding the mature IGF protein of the present invention may be genomic, cDNA, or synthetic DNA. The genes encoding the native forms of IGF have been sequenced, and several variants are well known in the art. . IGF-I and variants thereof can be produced in any number of ways that are well known in the art. For example, the IGF-I polypeptides can be isolated directly from blood, such as from serum or plasma, by known methods. See, for example, U.S. Patent No. 4,769,361; Svoboda *et al.* (1980) *Biochemistry* 19:790-797; Cornell and Boughdady (1982) *Prep. Biochem.* 12:57 and (1984) *Prep. Biochem.* 14:123; herein incorporated by reference. Alternatively, IGF-I can be synthesized chemically, by any of several techniques that are known to those skilled in the art. See, for example, Stewart and Young (1984) *Solid Phase Peptide Synthesis* (Pierce Chemical Company, Rockford, Illinois) and Barany and Merrifield (1980) *The Peptides: Analysis, Synthesis, Biology* (eds. Gross and Meienhofer) pp. 3-254, Vol. 2 (Academic Press, New York), for solid phase peptide synthesis techniques; and Bodansky (1984) *Principles of Peptide Synthesis* (Springer-Verlag, Berlin) and Gross and Meienhofer, eds. (1980) *The Peptides: Analysis, Synthesis, Biology*, Vol. 1, for classical solution synthesis; herein

5 incorporated by reference. The IGF-I polypeptides of the present invention can also
be chemically prepared by the method of simultaneous multiple peptide synthesis.
See, for example, Houghten (1985) *Proc. Natl. Acad. Sci. USA* 82:5131-5135; U.S.
Patent No. 4,631,211; herein incorporated by reference.

10 In this preferred embodiment comprising mature IGF-I, the C-terminal end of
the truncated α -factor secretion leader peptide sequence and the N-terminal end of the
native C-terminal propeptide sequence will terminate in a preferred processing site,
preferably a dibasic processing site that is specific for the KEX2 endopeptidase of *S.*
cerevisiae. The dipeptides can be any combination of the basic residues Lys and Arg,
more preferably a Lys-Arg dipeptide.

15 The nucleotide sequences of the present invention are useful for producing
biologically active mature heterologous proteins of interest in a yeast host cell when
operably linked to a yeast promoter. In this manner, the nucleotide sequences
encoding the hybrid precursor polypeptides of the invention are provided in
expression cassettes for introduction into a yeast host cell. These expression
20 cassettes will comprise a transcriptional initiation region linked to the nucleotide
sequence encoding the hybrid precursor polypeptide. Such an expression cassette is
provided with a plurality of restriction sites for insertion of the nucleotide sequence
to be under the transcriptional regulation of the regulatory regions. The expression
cassette may additionally contain selectable marker genes.

25 Such an expression cassette comprises in the 5' to 3' direction and operably
linked a yeast-recognized transcription and translation initiation region, a nucleotide
coding sequence for the hybrid precursor polypeptide comprising the sequence for the
mature protein of interest, and a yeast-recognized transcription and translation
termination region. By "operably linked" is intended expression of the coding
30 sequence for the hybrid precursor polypeptide is under the regulatory control of the
yeast-recognized transcription and translation initiation and termination regions.

By "yeast-recognized transcription and translation initiation and termination
regions" is intended regulatory regions that flank a coding sequence, in this case the
nucleotide sequence encoding the hybrid polypeptide sequence, and control
35 transcription and translation of the coding sequence in a yeast. These regulatory
regions must be functional in the yeast host. The transcription initiation region, the
yeast promoter, provides a binding site for RNA polymerase to initiate downstream

5 (3') translation of the coding sequence. The promoter may be a constitutive or inducible promoter, and may be native or analogous or foreign or heterologous to the specific yeast host. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. By foreign is intended that the transcription initiation region is not found in the native yeast of interest into which the
10 transcription initiation region is introduced.

Suitable native yeast promoters include, but are not limited to the wild-type α -factor promoter, as well as other yeast promoters. Preferably the promoter is selected from the list including promoters for the glycolytic enzymes phosphoglucosomerase, phosphofructokinase, phosphotrioseisomerase, phosphoglucosomutase, enolase,
15 pyruvate kinase (PyK), glyceraldehyde-3-phosphate dehydrogenase (GAP or GAPDH), alcohol dehydrogenase (ADH) (EPO Publication No. 284,044). See, for example, EPO Publication Nos. 120,551 and 164,556.

Synthetic hybrid promoters consisting of the upstream activator sequence of one yeast promoter, which allows for inducible expression, and the transcription
20 activation region of another yeast promoter also serve as functional promoters in a yeast host. Examples of hybrid promoters include ADH/GAP, where the inducible region of the ADH promoter is combined with the activation region of the GAP promoter (U.S. Patent Nos. 4,876,197 and 4,880,734). Other hybrid promoters using upstream activator sequences of either the ADH2, GAL4, GAL10, or PHO5 genes
25 combined with the transcriptional activation region of a glycolytic enzyme such as GAP or PyK are available in the art (EPO Publication No. 164,556). More preferably the yeast promoter is the inducible ADH/GAP hybrid promoter.

Yeast-recognized promoters also include naturally occurring non-yeast promoters that bind yeast RNA polymerase and initiate translation of the coding
30 sequence. Such promoters are available in the art. See, for example, Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Mercereau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109; Henikoff *et al.* (1981) *Nature* 283:835; and Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; herein incorporated by reference.

35 The termination regulatory region of the expression cassette may be native with the transcription initiation region, or may be derived from another source, providing that it is recognized by the yeast host. The termination regions may be

5 those of the native α -factor transcription termination sequence, or another yeast-recognized termination sequence, such as those for the glycolytic enzymes mentioned above. More preferably the transcription terminator is the *Mat- α* (α -factor) transcription terminator described in U.S. Patent No. 4,870,008.

10 The nucleotide sequences encoding the hybrid precursor polypeptides of the invention are provided in expression cassettes for expression in a yeast host. The cassette will include 5' and 3' regulatory sequences operably linked to the nucleotide sequence encoding the hybrid precursor polypeptide of interest. The cassette may also contain at least one additional nucleotide sequence of interest to be cotransformed into the yeast host. Alternatively, the additional nucleotide sequences
15 can be provided on another expression cassette. Where appropriate, the nucleotide sequence encoding the hybrid precursor polypeptide and any additional nucleotide sequences of interest may be optimized for increased expression in the transformed yeast. That is, these nucleotide sequences can be synthesized using yeast-preferred codons for improved expression. Methods are available in the art for synthesizing yeast-preferred nucleotide sequences of interest (see, for example, U.S. Patent Nos. 20 5,219,759 and 5,602,034).

Additional sequence modifications are known to enhance expression of nucleotide coding sequences in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals,
25 transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the nucleotide coding sequence is modified to avoid predicted hairpin secondary mRNA structures.

30 In preparing the expression cassette, the various nucleotide sequence fragments may be manipulated, so as to provide for the sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the nucleotide fragments or other manipulations may be involved to provide for convenient restriction sites, removal
35 of superfluous nucleotides, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, *e.g.*,

5 transitions and transversions, may be involved. See particularly Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, New York).

The expression cassettes of the present invention can be ligated into a replicon (e.g., plasmid, cosmid, virus, mini-chromosome), thus forming an expression vector that is capable of autonomous DNA replication in vivo. Preferably the replicon will be a plasmid. Such a plasmid expression vector will be maintained in one or more replication systems, preferably two replication systems, that allow for stable maintenance within a yeast host cell for expression purposes, and within a prokaryotic host for cloning purposes. Examples of such yeast-bacteria shuttle vectors include Yep24 (Botstein *et al.* (1979) *Gene* 8:17-24; pCl/l (Brake *et al.* (1984) *Proc. Natl. Acad. Sci. USA* 81:4642-4646), and Yrp17 (Stnichomb *et al.* (1982) *J. Mol. Biol.* 158:157).

Additionally, a plasmid expression vector may be a high or low copy number plasmid, the copy number generally ranging from about 1 to about 200. With high copy number yeast vectors, there will generally be at least 10, preferably at least 20, and usually not exceeding about 150 copies in a single host. Depending upon the heterologous protein selected, either a high or low copy number vector may be desirable, depending upon the effect of the vector and the foreign protein on the host. See, for example, Brake *et al.* (1984) *Proc. Natl. Acad. Sci. USA* 81:4642-4646. DNA constructs of the present invention can also be integrated into the yeast genome by an integrating vector. Examples of such vectors are known in the art. See, for example, Botstein *et al.* (1979) *Gene* 8:17-24.

The host chosen for expression of the heterologous proteins of the invention will preferably be a yeast. By "yeast" is intended ascosporogenous yeasts (Endomycetales), basidiosporogenous yeasts, and yeast belonging to the Fungi Imperfecti (Blastomycetes). The ascosporogenous yeasts are divided into two families, Spermophthoraceae and Saccharomycetaceae. The later is comprised of four subfamilies, Schizosaccharomycoideae (e.g., genus *Schizosaccharomyces*), Nadsonioideae, Lipomycoideae, and Saccharomycoideae (e.g., genera *Pichia*, *Kluyveromyces*, and *Saccharomyces*). The basidiosporogenous yeasts include the genera *Leucosporidium*, *Rhodospodium*, *Sporidiobolus*, *Filobasidium*, and *Filobasidiella*. Yeast belonging to the Fungi Imperfecti are divided into two families, Sporobolomycetaceae (e.g., genera *Sporobolomyces*, *Bullera*) and Cryptococcaceae

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5 (e.g., genus *Candida*). Of particular interest to the present invention are species within the genera *Pichia*, *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, and *Candida*. Of particular interest are the *Saccharomyces* species *S. cerevisiae*, *S. carlsbergensis*, *S. diastaticus*, *S. douglasii*, *S. kluyveri*, *S. norbensis*, and *S. oviformis*. Species of particular interest in the genus *Kluyveromyces* include *K. lactis*. Since the
10 classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Skinner *et al.*, eds. 1980) *Biology and Activities of Yeast* (Soc. App. Bacteriol. Symp. Series No. 9). In addition to the foregoing, those of ordinary skill in the art are presumably familiar with the biology of yeast and the manipulation of yeast genetics. See, for example, Bacila *et al.*, eds. (1978)
15 *Biochemistry and Genetics of Yeast*; Rose and Harrison, eds. (1987) *The Yeasts* (2nd ed.); Strathern *et al.*, eds. (1981) *The Molecular Biology of the Yeast Saccharomyces*; herein incorporated by reference.

The selection of suitable yeast and other microorganism hosts for the practice of the present invention is within the skill of the art. When selecting yeast hosts for
20 expression, suitable hosts may include those shown to have, inter alia, good secretion capacity, low proteolytic activity, and overall vigor. Yeast and other microorganisms are generally available from a variety of sources, including the Yeast Genetic Stock Center, Department of Biophysics and Medical Physics, University of California, Berkeley, California; and the American Type Culture Collection, Rockville,
25 Maryland.

Methods of introducing exogenous DNA into yeast hosts are well known in the art. There is a wide variety of ways to transform yeast. For example, spheroplast transformation is taught by Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1919-1933 and Stinchcomb *et al.*, EPO Publication No. 45,573; herein incorporated by
30 reference. Transformants are grown in an appropriate nutrient medium, and, where appropriate, maintained under selective pressure to insure retention of endogenous DNA. Where expression is inducible, growth can be permitted of the yeast host to yield a high density of cells, and then expression is induced. The secreted, mature heterologous protein can be harvested by any conventional means, and purified by
35 chromatography, electrophoresis, dialysis, solvent-solvent extraction, and the like.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

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The following examples further describe the construction of an expression vector comprising the nucleotide sequence encoding mature human PDGF-B in accordance with the disclosed invention. Examples demonstrating the use of this expression vector to produce biologically active mature PDGF-BB in a yeast host are also provided.

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Additional examples describe an expression vector comprising the nucleotide sequence encoding mature human IGF-I in accordance with the disclosed invention and demonstrate the use of this expression vector to produce biologically active mature IGF-I in a yeast host.

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Example 1: Plasmid Vector pAB24

The vector selected for expressing rhPDGF-BB, pAB24, is a yeast-bacteria shuttle vector. The plasmid is a chimera of sequences from pBR322, derived from several naturally occurring bacterial plasmids, and sequences of the endogenous *S. cerevisiae* 2- μ plasmid (Broach (1981) in *Molecular Biology of the Yeast Saccharomyces* (Cold Spring Harbor Press, New York), 1:445-470). It also encodes genes enabling selection in both *E. coli* and *S. cerevisiae* hosts. The pBR322 part of pAB24 includes the ampicillin resistance (Ap^r)-conferring gene encoding β -lactamase, as well as a gene conferring tetracycline resistance (Tc^r). These genes allow transformation of competent *E. coli* and selection of plasmid-containing bacteria. A unique BamHI cloning site, present in the gene encoding tetracycline resistance, is the site utilized for insertion of an expression cassette. The pBR322 portion of the vector also includes a ColE1-like replication origin enabling replication in *E. coli*. Two *S. cerevisiae* genes derived from YEp24 (Botstein *et al.* (1979) *Gene* 8:17-24), *URA3* and *leu2d*, enable selection in yeast host strains lacking either or both of these genes. The latter gene, *leu2d*, lacks a portion of the 5'-untranslated promoter region and requires high plasmid copy number for growth in leucine-deficient medium. This is necessary to achieve sufficient LEU2 protein expression for complementation of yeast strains lacking LEU2 (Erhart and Hollenberg (1983) *J. Bacteriol.* 156:625-635). The 2- μ sequences of pAB24 confer replication and partitioning of the expression plasmid in *S. cerevisiae*. Figure 1 shows a schematic map of plasmid pAB24 with key restriction sites and genetic elements. A description

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5 of the construction of pAB24 can be found in the European Patent Application publication EPO 0324 274 B1.

Three expression plasmids containing the *PDGF-B* gene, pYAGL7PB, pYL7PPB (also known as pYAGL7PPB), and PYJST400, were used to produce PDGF-BB in a yeast host. All of these expression vectors utilize pAB24 as the
10 plasmid into which the expression cassette comprising the *PDGF-B* gene was inserted.

Example 2: Construction of Expression Plasmid pYAGL7PB

15

General Description

Plasmid pYAGL7PB includes an expression cassette with the following features. Transcription is mediated by the inducible, hybrid yeast promoter
20 ADH/GAP. This promoter includes ADR2 transcription factor responsive sequences from the *S. cerevisiae ADH2* gene (Beier and Young (1982) *Nature* 300:724-728) and promoter sequences from the *S. cerevisiae* gene *TDH3*, encoding the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAP). The ADR2 transcription factor responsive sequences confer inducible gene transcription upon downstream
25 sequences. Induction is achieved by glucose depletion in the growth medium. Termination of transcription is mediated by the terminator derived from the *S. cerevisiae* mating factor type alpha (*Mata* α) gene (Brake *et al.* (1984) *Proc. Natl. Acad. Sci. USA* 81:4642-4646).

The cassette further includes an open reading frame encoding a truncated
30 *Mata* α sequence fused to a sequence encoding the human *PDGF-B* gene. The truncated α -factor leader mediates secretion of in-frame protein fusions. It is a derivative of *S. cerevisiae* α -factor leader, the product of the *Mata* α gene (Kurjan and Herskowitz (1982) *Cell* 30:933-943). A dibasic amino acid processing site is present at the truncated α -factor leader/PDGF-B junction to facilitate production of correctly
35 processed rhPDGF-BB polypeptide by yeast. Figure 2 shows a map of the pYAGL7PB expression cassette highlighting these features and the restriction enzyme sites relevant to the construction of this expression cassette. The nucleotide sequence and predicted amino acid sequence of the open reading frame encoding the truncated

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- 5 α -factor leader-PDGF-B primary translation product are given in SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Sequential Construction of pYAGL7PB

- 10 Following is a description of the sequential steps taken to construct this expression vector.

Construction of PDGF-B Synthetic Gene and Cloning into a Yeast Expression Vector

- 15 The synthetic gene encoding the partial dibasic processing site and rhPDGF-B (SEQ ID NOs: 3-4) was made from 17 overlapping oligonucleotides (SEQ ID NOs: 5-21) as described in Urdea *et. al.* (*Proc. Natl. Acad Sci. USA* 80 (1983):7461-7465). Ligation of the fragments results in an XbaI-SalI fragment, which was subsequently inserted into XbaI-SalI cut pPAG/ α F vector.

- 20 Plasmid pPAG/ α F is a pBR322 derivative with an expression cassette delineated by BamHI sites. The expression cassette includes the ADH/GAP hybrid promoter, as well as the open reading frame encoding the yeast α -factor leader (BamHI-XbaI), an XbaI-SalI gene fragment, and the *Mata* (α -factor) transcription terminator (SalI-BamHI). Substitution of an XbaI-SalI gene fragment (in-frame) capable of heterologous protein expression into this plasmid allows the expression
25 and secretion of the heterologous protein. The isolation of the yeast glyceraldehyde-3-phosphate (GAP) gene promoter, the origin of the *ADH2* component of the promoter, and the construction of a hybrid ADH/GAP promoter are described in U.S. Patent Nos. 4,876,197 and 4,880,734. The isolation of the yeast α -factor gene including the transcription terminator is described in U.S. Patent No. 4,870,008.

- 30 Upon dideoxy sequencing, the synthetic gene sequence was found to have a single base pair mutation, which was repaired by standard procedures. Plasmid pPAGBB-1 is the plasmid derived from pPAG/ α F that contains the correct synthetic PDGF-B (XbaI-SalI) gene.

- 35 Construction of Synthetic Truncated α -Factor Leader Gene with Dibasic Processing Site

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5 The truncated α -factor leader mediates secretion of in-frame hybrid polypeptides. It is a derivative of *S. cerevisiae* α -factor leader, the secretion leader for mating factor type alpha, the product of the *Mata* gene (Kurjan and Herskowitz (1982) *Cell* 30:933-943), and consists of the first 35 amino acids of the native leader. The construction and use of a truncated α -factor leader gene to mediate secretion is described in EPO Publication No. 0324 274 B1. Synthetic oligonucleotides encoding a comparable, partial (amino acids 8-35) truncated α -factor leader (L7) and part of the dibasic processing site were made from oligonucleotides given in SEQ. ID NO: 22 and and when assembled with the complementary strand shown in SEQ ID NO: 23 resulted in a PstI-BglII fragment with a 3' -ACGTC- and a 5' -CTAG- overhang to allow for convenient ligation into the expression cassette.

Construction of pAGL7PB

 The purpose of this construction was the substitution of the synthetic, partial truncated α -factor leader PstI-BglII gene fragment described above for most of the full-length α -factor leader in the PDGF-B expression cassette of pPAGBB-1. A 1.9 kb Pst I fragment including pBR322 sequences, the ADH/GAP hybrid promoter (marked at the 5' end by a BamHI site) and the 5' partial α -factor leader gene sequence (encoding the first seven amino acids of the native α -factor leader) was isolated from pPAGBB-1. It was ligated to kinased, annealed synthetic oligonucleotides 1.49/3°.40. Following digestion with BamHI, a partial expression cassette 5' fragment was obtained including sequences for the ADH/GAP hybrid promoter and the 5' portion of the truncated α -factor leader.

 Similarly, a BglII fragment containing the PDGF-B synthetic gene, the α -factor terminator (marked at the 3' end by a BamHI site) and pBR322 sequences was isolated from pPAGBB-1. It was ligated to kinased, annealed synthetic oligonucleotides 2.32/4°.50. Following digestion with BamHI, a partial expression cassette 3' fragment was obtained including sequences for the 3' portion of the truncated α -factor leader, PDGF-B, and the α -factor leader transcription terminator. The complete PDGF-B expression cassette was obtained following ligation of the 5' and 3' partial expression cassette gene fragments and digestion with BamHI. The BamHI expression cassette was cloned into the BamHI site of a pBR322-derived

5 vector (pBRΔEco-Sal) to give plasmid pAGL7PB. A map of the PDGF-B expression cassette in this plasmid is shown in Figure 2.

Construction of pYAGL7PB

The PDGF-B expression cassette of pAGL7PB was isolated by BamHI digestion and inserted into the BamHI site of the yeast-bacteria shuttle vector pAB24 described above. A yeast expression plasmid, pYAGL7PB, was isolated. A plasmid map of pYAGL7PB is shown in Figure 3. The nucleotide sequence of the complete expression cassette and the predicted amino acid sequence of the open reading frame (ORF) encoding the truncated α -factor leader-PDGF-B primary translation product are given in SEQ ID NO: 24 and SEQ ID NO: 25, respectively.

Expression Strain Identification: MB2-1(pYAGL7PB)

Expression plasmid pYAGL7PB was transformed into *S. cerevisiae* MB2-1 by standard procedures and prototrophic uracil colonies were selected. Individual colonies from independent transformants were screened for expression following inoculation of single colonies into medium that selects for leucine prototrophs. The medium also is high in glucose to keep expression of sequences under *ADR2* regulation (including the PDGF-B gene) repressed. Cultures were subsequently diluted and grown to confluence in low glucose medium lacking uracil. Cell-free culture supernatants were prepared and assayed for PDGF-BB by immunoactivity (ELISA) and by mitogenic activity on 3T3 cells. A high PDGF-BB expressing colony, MB2-1(pYAGL7PB #5), was identified.

Example 3: Construction of Expression Plasmid pYL7PPB

General Description

Plasmid pYL7PPB (also known as pYAGL7PPB) includes an expression cassette with the following features. Transcription initiation and termination is mediated by the inducible, hybrid yeast promoter ADH/GAP and the *Mata* transcriptional terminator described above. The gene further includes an open reading frame encoding a truncated yeast α -factor leader to mediate secretion of rhPDGF-BB. The propeptide sequence included in the expression construct is only the native N-terminal propeptide sequence; the native C-terminal propeptide sequence was not

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5 included in the construct. Inclusion of the N-terminal propeptide sequence resulted in enhanced expression of rhPDGF-BB, presumably because of improved folding. Dibasic processing sites at the truncated α -factor leader/N-terminal propeptide and N-terminal propeptide/PDGF-B junctions were included to facilitate production of correctly processed rhPDGF-BB polypeptide by yeast. Figure 4 shows a map of the

10 pYL7PPB expression cassette highlighting these features and the sites relevant to the construction of this expression cassette. The nucleotide sequence and predicted amino acid sequence of the open reading frame encoding the truncated α -factor leader-proPDGF-B primary translation product are shown in SEQ ID NO: 26 and SEQ ID NO: 27, respectively.

Sequential Construction of pYL7PPB

Source of rhPDGF-B cDNA

A cloned cDNA encoding native human preproPDGF-B, λ hPDGFb-17, was provided by collaborators Arne Östman and Carl Heldin. Isolation of the cDNA

20 encoding hPDGF-B was achieved using a cDNA library prepared from RNA isolated from a human clonal glioma cell line, U-343 MGa Cl 2 (Östman *et al.* (1988) *J. Biol. Chem.* 263:16202-16208).

Construction of pSV7d-PDGF A103-B1

Plasmid pSV7d-PDGF A103-B1 was the source of the N-terminal propeptide-PDGF-B cDNA. The plasmid was constructed as described below.

25

The 3 kb Eco RI PDGF-B cDNA insert from clone λ hPDGFb-17 was excised and cloned into the unique Eco RI site of the mammalian expression vector pSV7d to give plasmid phPDGF β -1 (also known as pSV7d-PDGF-B1).

A mammalian plasmid, pSV7d-PDGF A103- β 1, for the coexpression of both

30 PDGF-A and -B chains from their respective cDNAs, was constructed as follows. Plasmid phPDGF β -1 was digested with PstI under conditions favoring cleavage at one of the two plasmid PstI sites (desired single cleavage at site in ampicillin resistance gene of the pSV7d vector backbone) and ligated with PstI-digested pSV7d-PDGF-A103(D1). This latter plasmid is strictly analogous to the PDGF-B mammalian expression plasmid phPDGF β -1, except that it includes cDNA encoding the long,

35 211 amino acid form of the PDGF-A chain rather than the PDGF-B chain cDNA. This

28

5 plasmid contains a single PstI site in the ampicillin resistance gene of the pSV7d vector backbone.

Following transformation, bacterial colonies were screened for the presence of both PDGF-B and PDGF-A cDNA sequences with the respective or appropriately labeled EcoRI cDNA probes. Colonies positive for both PDGF-B and -A chain
10 sequences were further screened by EcoRI digestion of plasmid DNA, and plasmid pSV7d-PDGF A103-B1, having a predicted EcoRI pattern, was identified.

Mutagenesis of hPDGF-B cDNA

The PDGF-B cDNA was mutagenized: (1) to introduce a SacI site enabling introduction of the truncated α -factor secretion leader, and (2) to change the hPDGF-B
15 B cDNA sequence encoding dibasic amino acids Arg-Arg to encode Lys-Arg. This dibasic combination is more efficiently cleaved than Arg-Arg by the yeast dibasic processing enzyme KEX2 endopeptidase. The template for mutagenesis was prepared as follows.

The ~3kb EcoRI hPDGF-B cDNA was isolated from pSV7d-PDGF A103-B1
20 and inserted into the EcoRI site of pBR322 to give plasmid pPPB/6. The nucleotide sequence of the 2.7 kb PstI-EcoRI cDNA fragment was verified. The 0.9 kb PstI-NcoI cDNA fragment was inserted into the PstI-NcoI sites of M13 and the nucleotide sequence of the insert verified. A partial nucleotide sequence and the predicted amino acid sequence of the PDGF-B cDNA are given in SEQ ID NO: 28 and SEQ ID NO:
25 29, respectively.

A double mutagenesis of M13 PstI-NcoI PDGF-B cDNA fragment was performed by standard methods using the following primers. Primer 1 (SEQ ID NO: 30) introduces a SacI site; primer 2 (SEQ ID NO: 31) converts Arg-Arg to Lys-Arg at the propeptide/PDGF-B junction. Additional mutations are introduced to facilitate
30 detection of mutagenized sequences by hybridization with the labeled primer. No changes resulted in the primary amino acid sequence by primer 1 mutagenesis; only the Arg \Rightarrow Lys amino acid change resulted from primer 2 mutagenesis. Mutant hPDGF-B inserts were detected by hybridization with both primer 1 and 2 radiolabeled probes. DNA sequence was verified, and RF (double-stranded) plasmid
35 was prepared.

Construction of pL7PPB (pAGL7PPB)

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Essentially, the steps described below result in the substitution of the XhoI-SalI portion of the PDGF-B expression cassette in pAGL7PB encoding the C-terminal portion of the truncated α -factor leader, the Lys-Arg dibasic processing site and PDGF-B (Figure 2) with an XhoI-SalI gene fragment encoding the C-terminal portion of the truncated α -factor leader, a Lys-Arg dibasic processing site, the PDGF-B N-terminal propeptide, a Lys-Arg dibasic processing site, and PDGF-B. The sequences encoding the N-terminal PDGF-B propeptide and PDGF-B were derived from cDNA as described above. A map of the resulting expression cassette is shown in Figure 4.

A 447 bp SacI-SphI fragment including most of the proPDGF-B gene was isolated from the M13 RF containing the modified preproPDGF-B cDNA. Synthetic oligonucleotides, including sequences encoding the C-terminal part of truncated α factor leader, a Lys-Arg dibasic processing site, and the N-terminal portion of the PDGF-B propeptide (SEQ ID NOs: 32-33), were joined to give a fragment with a 3' SacI overhang. Synthetic oligonucleotides, Sph-Sal I/Sph-Sal II, including sequences encoding the last 14 amino acids of PDGF-B and stop codons were joined to give a SphI-SalI fragment (SEQ ID NOs: 34-35). These two sets of annealed oligonucleotides were ligated to the 447 bp SacI-SphI proPDGF gene fragment. This resulted in a gene fragment including sequences encoding the C-terminal part of truncated α -factor leader, a Lys-Arg dibasic processing site and proPDGF-B.

Synthetic oligonucleotides, including sequences encoding the middle amino acids of the truncated α -factor leader were joined resulting in a fragment with a 5' XhoI overhang (SEQ ID NOs: 32-33). This annealed oligonucleotide was ligated with pAGL7PB that had been cut with XhoI (unique site in pAGL7PB plasmid that is in the expression cassette, see Figure 2). Following oligonucleotide annealing, the modified plasmid was digested with SalI resulting in loss of the pAGL7PB XhoI-SalI fragment and resulting in a vector/gene fragment.

The final step in the construction of the PDGF-B expression cassette was the ligation of the gene fragment into the vector/gene fragment to give plasmid pL7PPB (pAGL7PPB), as shown in Figure 5. The PstI-BamHI insert fragment was isolated and nucleotide sequencing confirmed that the desired construction had been obtained. A map of the PDGF-B expression cassette in pL7PPB is shown in Figure 4.

Construction of pYL7PPB (pYAGL7PPB)

5 The PDGF-B expression cassette of pL7PPB³⁰ was isolated following BamHI digestion and inserted into the BamHI site of the yeast shuttle vector pAB24, described above, resulting in yeast expression plasmid pYL7PPB. A map of pYL7PPB is shown in Figure 6. The nucleotide sequence of the complete expression cassette and the predicted amino acid sequence of the open reading frame (ORF) encoding truncated α -factor leader-Lys-Arg-proPDGF-B are given in SEQ ID NO: 36 and SEQ ID NO: 37, respectively. The complete nucleotide sequence of yeast expression plasmid pYL7PPB has been determined.

15 **Expression Strain Identification: MB2-1(pYL7PPB)**

 Expression plasmid pYL7PPB was transformed into *S. cerevisiae* MB2-1 by standard procedures and plasmid-harboring, uracil prototrophs were selected as isolated colonies. Individual colonies from independent transformants were screened for expression following inoculation of isolated colonies into growth medium that selects for leucine prototrophs. The medium also is high in glucose to keep expression of sequences under *ADR2* regulation (including the PDGF-B gene) repressed. Cultures were subsequently diluted and grown to confluence in low glucose, selective growth medium lacking uracil. Cell-free supernatants were assayed for PDGF-BB by immunoactivity (ELISA) and by mitogenic activity on 3T3 cells. Frozen stocks were prepared of several transformants exhibiting consistently high levels of expression. Following repeated testing, the transformant exhibiting, on average, the highest expression of PDGF-BB, MB2-1 (pYL7PPB #22) was selected.

Example 4: Expression Plasmid pYJST400

30 The Lys-Arg dibasic processing site between the α -factor leader sequence and the N-terminal propeptide was eliminated from expression plasmid pYL7PPB by *in vitro* mutagenesis to construct expression plasmid pYJST400. Thus pYJST400 has a single dibasic processing site, which resides at the propeptide/PDGF-B junction. Elimination of this first processing site was done to determine its relative effect on secretion of rhPDGF-BB from yeast as mediated by the α -factor leader peptide.

35 **Example 5: Expression of Recombinant Human PDGF-BB**

These results indicate that the presence of the native N-terminal propeptide enhances secretion of biologically active mature rhPDGF-BB when flanked by preferred processing sites that have been modified for improved recognition by a proteolytic enzyme of the yeast host cell. Thus, cleavage at the leader/propeptide junction, as well as at the propeptide/PDGF-B junction, apparently facilitates the proper folding and/or processing and/or transport of the pro-PDGF-B, resulting in enhanced secretion of mature rhPDGF-BB.

TABLE 1

Secretion of rhPDGF-BB in Yeast						
Strain	Plasmid	Translation Product	N _(t)	Mitogen (mean, std. dev.)		ELISA (mean, std. dev.)
				$\mu\text{g/L}$	$\mu\text{g/L/OD}_{650}$	$\mu\text{g/L}$
MB2-1	pYAGL7PB	$\alpha\text{F}_{\text{L1-35}}\text{PDGF-B}^*$	16	2,105 (375)	605 (135)	2,147 (686)
MB2-1	pYL7PPB	$\alpha\text{F}_{\text{L1-35pro}}\text{PDGF-B}$	22	7,163 (1,323)	1,427 (305)	4,662 (938)
MB2-1	pYJST400	$\alpha\text{F}_{\text{L1-35}}\Delta\text{KRproPDGF-B}$	10	2,584 (445)	549 (113)	2,220 (410)
						$\mu\text{g/L/OD}_{650}$
						604 (166)
						907 (191)
						472 (104)

* $\alpha\text{F}_{\text{L1-35}}\text{PDGF-B}$ = a truncated α -factor leader consisting of the N-terminal amino acids 1-35 fused in-frame with mature human PDGF-B. A single processing site (KEX_2) separates the leader sequence from the mature PDGF-B sequence.

$\alpha\text{F}_{\text{L1-35pro}}$ = a truncated α -factor leader consisting of the N-terminal amino acids 1-35 plus the native N-terminal propeptide for human PDGF-B fused in-frame with mature human PDGF-B. KEX_2 processing sites separate the leader sequence from the N-terminal propeptide sequence (KEX_1) and the N-terminal sequence from the mature PDGF-B sequence (KEX_2).

$\alpha\text{F}_{\text{L1-35}}\Delta\text{KRproPDGF-B}$ = a truncated α -factor leader consisting of the N-terminal amino acids 1-35 plus the native N-terminal propeptide for human PDGF-B fused in-frame with mature human PDGF-B. The KEX_1 processing site was removed (ΔKR) from between the leader sequence and the N-terminal propeptide sequence.

5 **Example 6: Human Foreskin Fibroblast (HFF)****Mitogen Assay for PDGF**

Human foreskin fibroblast stocks were stored frozen; freezing was at passage 13. Prior to use, HFF were thawed and then grown in T75 flasks until confluent, which usually occurred at 5-7 days. Growth medium contained Dulbecco's Modified Eagles Medium (DMEM), 20% fetal bovine serum (FBS), 1 mM sodium pyruvate, 300 µg/ml L-glutamine, 100U/ml penicillin, and 100 µg/ml streptomycin. Cells were incubated at 37 C in humidified 7% CO₂, 93% air atmosphere. At confluency, cells were passaged by rinsing the monolayer with phosphate buffered saline (PBS) lacking C²⁺ and Mg²⁺, dissociating them in trypsin containing EDTA, and diluting them with growth medium. Cells were passaged no more than 8 times after thawing.

To assay for PDGF, HFFs were plated as follows. The cells were rinsed and dissociated with trypsin as above. The trypsinized cells were pelleted and resuspended to a concentration of 1 x 10⁵ cells/ml in medium similar to growth medium, except that 5% FBS replaced 20% FBS; 100 µl of suspension was dispensed into each well of a 96-well microtiter plate; and then the cells were incubated 5-6 days under the above described conditions.

PDGF in the sample was determined by monitoring ³H-thymidine incorporation into HFF DNA stimulated by PDGF. Samples were added to the wells containing HFF monolayers, and the assay plates incubated as above for 18 hours. The HFF cultures were then pulsed with [Methyl-³H]thymidine (10 µC/ml final concentration, 1 µC/well) at 37 C under the above described incubation conditions for 8 hours. After incubation, the cells were rinsed with PBS and fixed. Fixing was by incubation with 5% trichloroacetic acid (TCA) and then 100% methanol for 15 minutes, followed by drying in air. The cells were then solubilized with 0.3N NaOH and then counted in a liquid scintillation counter.

Control samples were treated as the samples described above and were prepared as follows. For positive controls, PDGF, purchased from PDGF, Inc., was dissolved to a final concentration of 100 ng/ml in DMEM containing 10 mg/ml BSA. A standard curve was prepared; the first point was 10 ng/ml, the remaining points were 2-fold serial dilutions. Each dilution was tested in triplicate. Negative controls, which lacked both sample and control PDGF, were also run.

34

Example 7: Expression Plasmids pYLUI

Plasmid pYLUIGF24 includes an expression cassette with the hybrid yeast promoter ADH/GAP and *Mat α* factor leader sequences fused to a sequence encoding the human IGF-I-A gene. This sequence was synthetically derived using yeast preferred codons. A dibasic amino acid processing site is present at the α -factor leader/IGF-I-A junction. The nucleotide sequence and predicted amino acid sequence of the open reading frame encoding α -factor leader/IGF-I-A primary translation product are given in SEQ ID NO: 38 and SEQ ID NO: 39, respectively.

Plasmid pYLUIGF34 differs from pYLUIGF24 only in its open reading frame. This cassette includes an open reading frame encoding a full length *Mat α* factor leader sequence fused to a sequence encoding the human IGF-I-A gene with its C-terminal prosequence. Dibasic amino acid processing sites are present at the α -factor leader/IGF-I-A and IGF-I-A/IGF-I-A prosequence junctions. The nucleotide sequence and predicted amino acid sequence of the open reading frame encoding α -factor leader-proIGF-I-A primary translation product are given in SEQ ID NO: 40 and SEQ ID NO: 41, respectively.

Both of these plasmids were generated by inserting the respective expression cassette into the unique BamHI cloning site of the yeast shuttle vector pAB24 as described above.

Example 8: Expression of Recombinant Human PDGF-BB

Recombinant human IGF-I-A is produced by a strain of the yeast *Saccharomyces cerevisiae*, genetically modified with a multicopy yeast expression plasmid that includes a gene encoding human IGF-I-A. Yeast expression plasmids pYLUIGF24 and pYLUIGF34 were transformed into a yeast strain by procedures previously mentioned.

Western blot data indicated that properly processed IGF-IA protein was obtained with the prosequence, modified KEX2 processing site, and a yeast secretion leader.

35

5 All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

10 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Tekamp-Olson, Patricia
- (ii) TITLE OF INVENTION: METHOD FOR EXPRESSION OF HETEROLOGOUS PROTEINS IN YEAST
- (iii) NUMBER OF SEQUENCES: 41
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Bell Seltzer IP Group of Alston & Bird, LLP
 - (B) STREET: 3605 Glenwood Ave. Suite 310
 - (C) CITY: Raleigh
 - (D) STATE: NC
 - (E) COUNTRY: US
 - (F) ZIP: 27622
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Spruill, W. Murray
 - (B) REGISTRATION NUMBER: 32,943
 - (C) REFERENCE/DOCKET NUMBER: 5784-4
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 919 420 2202
 - (B) TELEFAX: 919 881 3175

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 444 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Chimeric nucleic acid"
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens/Saccharomyces cerevisiae
- (ix) FEATURE:
 - (A) NAME/KEY: CDS

(B) LOCATION: 1...441

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(ix) FEATURE:
      (A) NAME/KEY: misc_feature
      (B) LOCATION: 1..111
      (D) OTHER INFORMATION: /function= "mediates secretion of
proteins"
/product= "yeast alpha factor leader peptide"
/standard_name= "alpha factor signal/leader
sequence"
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(ix) FEATURE:
      (A) NAME/KEY: mat_peptide
      (B) LOCATION: 112..441
      (D) OTHER INFORMATION: /product= "rhPDGF-B protein"
/standard name= "rhPDGF-B"
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG Met -37	AGA Arg -35	TTT Phe	CCT Pro	TCA Ser	ATT Ile	TTT Phe	ACT Thr	GCA Ala	GTT Val	TTA Leu	TTC Phe	GCA Ala	GCC Ala	TCG Ser	AGC Ser	48
GCA Ala	TTA Leu -20	GCT Ala	GCT Ala	CCA Pro	GTC Val	AAC Asn -15	ACT Thr	ACA Thr	ACA Thr	GAA Glu	GAT Asp -10	GAA Glu	ACG Thr	GCA Ala	CAA Gln	96
ATT Ile -5	CCG Pro	GCT Ala	AAA Lys	AGA Arg	TCT Ser 1	TTG Leu	GGT Gly	TCT Ser	TTG Leu 5	ACT Thr	ATC Ile	GCT Ala	GAA Glu	CCA Pro 10	GCT Ala	144
ATG Met	ATC Ile	GCT Ala	GAA Glu 15	TGT Cys	AAG Lys	ACT Thr	AGA Arg	ACT Thr	GAA Glu 20	GTT Val	TTC Phe	GAA Glu 25	ATC Ile	TCC Ser	AGA Arg	192
AGA Arg	TTG Leu 30	ATC Ile	GAC Asp	AGA Arg	ACT Thr	AAC Asn	GCT Ala 35	AAC Asn	TTC Phe	TTG Leu	GTT Val	TGG Trp 40	CCA Pro	CCA Pro	TGT Cys	240
GTT Val	GAA Glu 45	GTT Val	CAA Gln	AGA Arg	TGT Cys	TCT Ser 50	GGT Gly	TGT Cys	TGT Cys	AAC Asn	AAC Asn	AGA Arg 55	AAG Asn	GTT Val	CAA Gln	288
TGT Cys 60	AGA Arg	CCA Pro	ACT Thr	CAA Gln	GTT Val 65	CAA Gln	TTG Leu	AGA Arg	CCA Pro	GTT Val 70	CAA Gln	GTT Val	AGA Arg	AAG Lys	ATC Ile 75	336
GAA Glu	ATC Ile	GTT Val	AGA Arg	AAG Lys 80	AAG Lys	CCA Pro	ATC Ile	TTC Phe	AAG Lys 85	AAG Lys	GCT Ala	ACT Thr	GTT Val	ACT Thr 90	TTG Leu	384
GAA Glu	GAC Asp	CAC His	TTG Leu 95	GCT Ala	TGT Cys	AAG Lys	TGT Cys	GAA Glu 100	ACT Thr	GTC Val	GCC Ala	GCT Ala	GCC Ala	AGG Arg	CCA Pro	432

GTT ACT TAA TAG
 Val Thr *
 110

444

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 147 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Arg	Phe	Pro	Ser	Ile	Phe	Thr	Ala	Val	Leu	Phe	Ala	Ala	Ser	Ser	-37	-35	-30	-25
Ala	Leu	Ala	Ala	Pro	Val	Asn	Thr	Thr	Thr	Glu	Asp	Glu	Thr	Ala	Gln	-20	-15	-10	
Ile	Pro	Ala	Lys	Arg	Ser	Leu	Gly	Ser	Leu	Thr	Ile	Ala	Glu	Pro	Ala	-5	1	5	10
Met	Ile	Ala	Glu	Cys	Lys	Thr	Arg	Thr	Glu	Val	Phe	Glu	Ile	Ser	Arg	15	20	25	
Arg	Leu	Ile	Asp	Arg	Thr	Asn	Ala	Asn	Phe	Leu	Val	Trp	Pro	Pro	Cys	30	35	40	
Val	Glu	Val	Gln	Arg	Cys	Ser	Gly	Cys	Cys	Asn	Asn	Arg	Asn	Val	Gln	45	50	55	
Cys	Arg	Pro	Thr	Gln	Val	Gln	Leu	Arg	Pro	Val	Gln	Val	Arg	Lys	Ile	60	65	70	75
Glu	Ile	Val	Arg	Lys	Lys	Pro	Ile	Phe	Lys	Lys	Ala	Thr	Val	Thr	Leu	80	85	90	
Glu	Asp	His	Leu	Ala	Cys	Lys	Cys	Glu	Thr	Val	Ala	Ala	Ala	Arg	Pro	95	100	105	
Val	Thr	*														110			

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 352 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTCTAGATAA AAGATCTTTG GGTTCCTTTGA CTATCGCTGA ACCAGCTATG ATCGCTGAAT	60
GTAAGACTAG AACTGAAGTT TTCGAAATCT CCAGAAGATT GATCGACAGA ACTAACGCTA	120
ACTTCTTGGT TTGGCCACCA TGTGTTGAAG TTCAAAGATG TTCTGGTTGT TGTAACAACA	180
GAAACGTTCA ATGTAGACCA ACTCAAGTTC AATTGAGACC AGTTCAAGTT AGAAAGATCG	240
AAATCGTTAG AAAGAAGCCA ATCTTCAAGA AGGCTACTGT TACTTTGGAA GACCACTTGG	300
CTTGTAAGTG TGAAACTGTT GCTGGTGCTA GACCAGTTAC TTAATAGCGT CG	352

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 352 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Complementing strand to the preceding SEQ ID NO:, listed to show the terminal overhangs produced upon assembly."

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCGACGACGC TATTAAGTAA CTGGTCTAGC AGCAGCAACA GTTTCACACT TACAAGCCAA	60
GTGGTCTTCC AAAGTAACAG TAGCCTTCTT GAAGATTGGC TTCTTTCTAA CGATTTTCGAT	120
CTTTCTAACT TGAAGTGGTC TCAATTGAAC TTGAGTTGGT CTACATTGAA CGTTTCTGTT	180
GTTACAACAA CCAGAACATC TTTGAACTTC AACACATGGT GGCCAAACCA AGAAGTTAGC	240
GTTAGTTCTG TCGATCGAAT CTTCTGGAGA TTTCGAAAAC TTAGTTCTAG TCTTACATTC	300
AGCGATCATA GCTGGTTCAG CGATAGTCAA AGAACCCAAA GATCTTTTAT CT	352

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCTAGATAA AAGATCTTTG GGTCTTTGA CTATCGCTGA ACCA

44

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCTATGATCG CTGAATGTAA GACTAGAACT GAAGTTTTTCG AAATC

45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCAGAAGAT TGATCGACAG AACTAACGCT AACTTCTTGG TTTGG

45

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCACCATGTG TTGAAGTTCA AAGATGTTCT GGTGTTGTA ACAAC

45

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGAAACGTTC AATGTAGACC AACTCAAGTT CAATTGAGAC CAGTT

45

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAAGTTAGAA AGATCGAAAT CGTTAGAAAG AAGCCAATCT TCAAG

45

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AAGGCTACTG TTACTTTGGA AGACCACTTG GCTTGTAAGT GTGA

44

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AACTGTTGCT GGTGCTAGAC CAGTTACTTA ATAGCGTCG

39

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCTATTTTCT AGAAACCC

18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AAGAACTGA TAGCGACTTG GTCGATACTA GCGACTTACA TTCTG

45

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATCTTGACTT CAAAAGCTTT AGAGGTCTTC TAACTAGCTG TCTTG

45

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATTGCGATTG AAGAACCAAA CCGGTGGTAC ACAACTTCAA GTTTC

45

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TACAAGACCA ACAACATTGT TGTCTTTGCA AGTTACATCT GGTTC

45

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGTTCAAGTT AACTCTGGTC AAGTTCAATC TTTCTAGCTT TAGCA

45

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 44 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATCTTTCTTC GGTAGAAGT TCTCCGATG ACAATGAAAC CTTC

44

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 44 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGGTGAACCG AACATTCACA CTTTGACAAC GACGACGATC TGGT

44

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAATGAATTA TCGCAGCAGC T

21

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Assembled synthetic oligonucleotides resulting in a truncated alpha factor mating pheromone leader sequence."

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Synthetic (derived from *Saccharomyces cerevisiae*)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TTTTATTTCGC AGCCTCGAGC GCATTAGCTG CTCCAGTCAA CACTACAACA GAAGATGAAA

60

CGGCACAAAT TCCGGCTAAA A

81

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 90 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "This sequence is the complementing strand of SEQ ID NO:1. It is submitted to illustrate the two terminal overhangs produced after assembly."

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Synthetic (derived from *Saccharomyces cerevisiae*)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GATCTTTTAG CCGGAATTTG TGCCGTTTCA TCTTCTGTTG TAGTGTTGAC TGGAGCAGCT 60
 AATGCGCTCG AGGCTGCGAA TAAAACTGCA 90

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1845 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Synthetic chimera"
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens/Saccharomyces cerevisiae
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1115..1558
- (ix) FEATURE:
 - (A) NAME/KEY: promoter
 - (B) LOCATION: 1..1114
 - (D) OTHER INFORMATION: /standard_name= "ADH/GAP promoter"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION: 1115..1225
 - (D) OTHER INFORMATION: /function= "mediates secretion of rhPDGF-B"
- /product= "truncated alpha factor leader/signal peptide"
- /standard_name= "alpha factor leader/signal sequence"
- (ix) FEATURE:
 - (A) NAME/KEY: mat_peptide
 - (B) LOCATION: 1226..1558
 - (D) OTHER INFORMATION: /product= "rhPDGF-B peptide"
- /standard_name= "rhPDGF-B"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGATCCTTCA ATATGCGCAC ATACGCTGTT ATGTTCAAGG TCCCTTCGTT TAAGAACGAA 60
 AGCGGTCTTC CTTTGTAGGG ATGTTTCAAG TTGTTCAAAT CTATCAAATT TGCAAATCCC 120
 CAGTCTGTAT CTAGCTAGAT ATACCAATGG CAACTGAGC ACAACAATAC CAGTCCGGAT 180
 CAACTGGCAC CATCTCTCCC GTAGTCTCAT CTAATTTTTC TTCCGGATGA GGTTCAGAT 240
 ATACCGCAAC ACCTTTATTA TGGTTTCCCT GAGGGAATAA TAGAATGTCC CATTCGAAAT 300
 CACCAATTCT AAACCTGGGC GAATTGTATT TCGGGTTTGT TAACTCGTTC CAGTCAGGAA 360

TGTTCCACGT GAAGCTATCT TCCAGCAAAG TCTCCACTTC TTCATCAAAT TGTGGGAGAA	420
TACTCCCAAT GCTCTTATCT ATGGGACTTC CGGGAAACAC AGTACCGATA CTTCCCAATT	480
CGTCTTCAGA GCTCATTGTT TGTTTGAAGA GACTAATCAA AGAATCGTTT TCTCAAAAAA	540
ATTAATATCT TAACTGATAG TTTGATCAAA GGGGCAAAAC GTAGGGGCAA ACAAACGGAA	600
AAATCGTTTC TCAAATTTTC TGATGCCAAG AACTCTAACC AGTCTTATCT AAAAATTGCC	660
TTATGATCCG TCTCTCCGGT TACAGCCTGT GTAAGTATT AATCCTGCCT TTCTAATCAC	720
CATTCTAATG TTTTAATTAA GGGATTTTGT CTTCATTAAC GGCTTTTCGCT CATAAAAATG	780
TTATGACGTT TTGCCCCGAG GCGGGAAACC ATCCACTTCA CGAGACTGAT CTCCTCTGCC	840
GGAACACCGG GCATCTCCAA CTTATAAGTT GGAGAAATAA GAGAATTTCA GATTGAGAGA	900
ATGAAAAAAA AAAACCCCTGA AAAAAAAGGT TGAAACCAGT TCCCTGAAAT TATTCCTCA	960
CTTGACTAAT AAGTATATAA AGACGGTAGG TATTGATTGT AATTCTGTAA ATCTATTTCT	1020
TAAACTTCTT AAATTCTACT TTTATAGTTA GTCTTTTTTT TAGTTTTAAA ACACCAAGAA	1080
CTTAGTTTCG AATAAACACA CATAAACAAA CACC ATG AGA TTT CCT TCA ATT	1132
Met Arg Phe Pro Ser Ile	
-37 -35	
TTT ACT GCA GTT TTA TTC GCA GCC TCG AGC GCA TTA GCT GCT CCA GTC	1180
Phe Thr Ala Val Leu Phe Ala Ala Ser Ser Ala Leu Ala Ala Pro Val	
-30 -25 -20	
AAC ACT ACA ACA GAA GAT GAA ACG GCA CAA ATT CCG GCT AAA AGA TCT	1228
Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln Ile Pro Ala Lys Arg Ser	
-15 -10 -5 1	
TTG GGT TCT TTG ACT ATC GCT GAA CCA GCT ATG ATC GCT GAA TGT AAG	1276
Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu Cys Lys	
5 10 15	
ACT AGA ACT GAA GTT TTC GAA ATC TCC AGA AGA TTG ATC GAC AGA ACT	1324
Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp Arg Thr	
20 25 30	
AAC GCT AAC TTC TTG GTT TGG CCA CCA TGT GTT GAA GTT CAA AGA TGT	1372
Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln Arg Cys	
35 40 45	
TCT GGT TGT TGT AAC AAC AGA AAC GTT CAA TGT AGA CCA ACT CAA GTT	1420
Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr Gln Val	
50 55 60 65	
CAA TTG AGA CCA GTT CAA GTT AGA AAG ATC GAA ATC GTT AGA AAG AAG	1468
Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg Lys Lys	
70 75 80	

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CCA ATC TTC AAG AAG GCT ACT GTT ACT TTG GAA GAC CAC TTG GCT TGT      1516
Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu Ala Cys
           85                      90                      95

AAG TGT GAA ACT GTC GCC GCT GCC AGG CCA GTT ACT TAA TAG      1558
Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr * *
           100                      105                      110

CGTCGTCGAC TTTGTTCCCA CTGTACTTTT AGCTCGTACA AAATACAATA TACTTTTCAT      1618

TTCTCCGTAA ACAACATGTT TTCCCATGTA ATATCCTTTT CTATTTTTCG TTCCGTTACC      1678

AACTTTACAC ATACTTTTATA TAGCTATTCA CTTCTATACA CTAAAAAACT AAGACAATTT      1738

TAATTTTGCT GCCTGCCATA TTTCAATTTG TTATAAATTC CTATAATTTA TCCTATTAGT      1798

AGCTAAAAAA AGATGAATGT GAATCGAATC CTAAGAGAAT TCGGATC      1845

```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 148 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
-37      -35                      -30                      -25

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
-20                      -15                      -10

Ile Pro Ala Lys Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala
-5              1              5              10

Met Ile Ala Glu Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg
      15              20              25

Arg Leu Ile Asp Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys
      30              35              40

Val Glu Val Gln Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln
      45              50              55

Cys Arg Pro Thr Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile
      60              65              70              75

Glu Ile Val Arg Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu
      80              85              90

Glu Asp His Leu Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro
      95              100              105

```

Val Thr * *
110

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 621 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "This construct is a chimeric nucleic acid that contains a truncated yeast alpha factor leader sequence linked to the human PDGF prosequence and the human rhPDGF-B gene(cDNA)."
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae/Homo sapiens
- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..621
- (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 25..105
(D) OTHER INFORMATION: /function= "Mediates secretion of human rhPDGF-B"
/product= "Saccharomyces cerevisiae alpha-factor leader/signal sequence"
- (ix) FEATURE:
(A) NAME/KEY: transit_peptide
(B) LOCATION: 112..288
(D) OTHER INFORMATION: /function= "Mediates protein transport"
/product= "human PDGF propeptide"
- (ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 289..621
(D) OTHER INFORMATION: /product= "human PDGF-B peptide"
/standard_name= "rhPDGF-B"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATG AGA TTT CCT TCA ATT TTT ACT GCA GTT TTA TTC GCA GCC TCG AGC	48
Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser	
-96 -95 -90 -85	
GCA TTA GCT GCT CCA GTC AAC ACT ACA ACA GAA GAT GAA ACG GCA CAA	96
Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln	
-80 -75 -70 -65	

ATT CCG GCT AAA AGA GAC CCC ATT CCC GAG GAG CTC TAC GAG ATG CTG	144
Ile Pro Ala Lys Arg Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met Leu	
-60 -55 -50	
AGT GAC CAC TCG ATC CGC TCC TTT GAT GAT CTC CAA CGC CTG CTG CAC	192
Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu His	
-45 -40 -35	
GGA GAC CCC GGA GAG GAA GAT GGG GCC GAG TTG GAC CTG AAC ATG ACC	240
Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met Thr	
-30 -25 -20	
CGC TCC CAC TCT GGA GGC GAG CTG GAG AGC TTG GCT CGG GGG AAG AGG	288
Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Lys Arg	
-15 -10 -5	
AGC CTG GGT TCC CTG ACC ATT GCT GAG CCG GCC ATG ATC GCC GAG TGC	336
Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu Cys	
1 5 10 15	
AAG ACG CGC ACC GAG GTG TTC GAG ATC TCC CGG CGC CTC ATA GAC CGC	384
Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp Arg	
20 25 30	
ACC AAC GCC AAC TTC CTG GTG TGG CCG CCC TGT GTG GAG GTG CAG CGC	432
Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln Arg	
35 40 45	
TGC TCC GGC TGC TGC AAC AAC CGC AAC GTG CAG TGC CGC CCC ACC CAG	480
Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr Gln	
50 55 60	
GTG CAG CTG CGA CCT GTC CAG GTG AGA AAG ATC GAG ATT GTG CGG AAG	528
Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg Lys	
65 70 75 80	
AAG CCA ATC TTT AAG AAG GCC ACG GTG ACG CTG GAA GAC CAC CTG GCA	576
Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu Ala	
85 90 95	
TGC AAG TGT GAG ACA GTG GCA GCT GCA CGG CCT GTG ACC TAA TAG	621
Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr * *	
100 105 110	

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 207 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
-96 -95                               -90                     -85

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
-80                               -75                     -70                     -65

Ile Pro Ala Lys Arg Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met Leu
                               -60                     -55                     -50

Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu His
                               -45                     -40                     -35

Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met Thr
-30                               -25                     -20

Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Lys Arg
-15                               -10                     -5

Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu Cys
 1                               5                     10                     15

Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp Arg
                20                     25                     30

Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln Arg
                35                     40                     45

Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr Gln
 50                               55                     60

Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg Lys
 65                               70                     75                     80

Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu Ala
                85                     90                     95

Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr * *
                100                     105                     110

```

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1320 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Synthetic chimera"
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens/Saccharomyces cerevisiae
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 454..1179

(ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION: 454..519
 (D) OTHER INFORMATION: /product= "PDGF-B prepeptide"
 /standard_name= "PDGF-B presequence"

(ix) FEATURE:
 (A) NAME/KEY: transit_peptide
 (B) LOCATION: 455..696
 (D) OTHER INFORMATION: /function= "mediates protein transport"
 /product= "PDGF-B propeptide"
 /standard_name= "PDGF-B prosequence"

(ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 697..1023
 (D) OTHER INFORMATION: /product= "rhPDGF-B peptide"
 /standard_name= "rhPDGF-B"

(ix) FEATURE:
 (A) NAME/KEY: transit_peptide
 (B) LOCATION: 1024..1179
 (D) OTHER INFORMATION: /function= "mediates protein transport"
 /product= "PDGF-B propeptide"
 /standard_name= "PDGF-B prosequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAATTCCTCCAG AAAATGTTGC AAAAAAGCTA AGCCGGCGGG CAGAGGAAAA CGCCTGTAGC	60
CGGCGAGTGA AGACGAACCA TCGACTGCCG TGTTCTTTT CCTCTTGGAG GTTGGAGTCC	120
CCTGGGCGCC CCCACACGGC TAGACGCCTC GGCTGGTTTCG CGACGCAGCC CCCCAGCCGT	180
GGATGCTGCA CTCGGGCTCG GGATCCGCCC AGGTAGCGGC CTCGGACCCA GGTCCTGCGC	240
CCAGGTCCTC CCCTGCCCCC CAGCGACGGA GCCGGGGCCG GGGGCGGCGG CGCCGGGGGC	300
ATGCGGGTGA GCCGCGGCTG CAGAGGCCTG AGCGCCTGAT CGCCGCGGAC CCGAGCCGAG	360
CCCACCCCCC TCCCCAGCCC CCCACCCTGG CCGCGGGGGC GGCGCGCTCG ATCTACGCGT	420
TCGGGGCCCC GCGGGGCCGG GCGCGGAGTC GGC ATG AAT CGC TGC TGG GCG CTC	474
Met Asn Arg Cys Trp Ala Leu	
-81 -80 -75	
TTC CTG TCT CTC TGC TGC TAC CTG CGT CTG GTC AGC GCC GAG GGG GAC	522
Phe Leu Ser Leu Cys Tyr Leu Arg Leu Val Ser Ala Glu Gly Asp	
-70 -65 -60	
CCC ATT CCC GAG GAG CTT TAT GAG ATG CTG AGT GAC CAC TCG ATC CGC	570
Pro Ile Pro Glu Glu Leu Tyr Glu Met Leu Ser Asp His Ser Ile Arg	
-55 -50 -45	

TCC TTT GAT GAT CTC CAA CGC CTG CTG CAC GGA GAC CCC GGA GAG GAA	618
Ser Phe Asp Asp Leu Gln Arg Leu Leu His Gly Asp Pro Gly Glu Glu	
-40 -35 -30	
GAT GGG GCC GAG TTG GAC CTG AAC ATG ACC CGC TCC CAC TCT GGA GGC	666
Asp Gly Ala Glu Leu Asp Leu Asn Met Thr Arg Ser His Ser Gly Gly	
-25 -20 -15	
GAG CTG GAG AGC TTG GCT CGT GGA AGA AGG AGC CTG GGT TCC CTG ACC	714
Glu Leu Glu Ser Leu Ala Arg Gly Arg Arg Ser Leu Gly Ser Leu Thr	
-10 -5 1 5	
ATT GCT GAG CCG GCC ATG ATC GCC GAG TGC AAG ACG CGC ACC GAG GTG	762
Ile Ala Glu Pro Ala Met Ile Ala Glu Cys Lys Thr Arg Thr Glu Val	
10 15 20	
TTC GAG ATC TCC CGG CGC CTC ATA GAC CGC ACC AAC GCC AAC TTC CTG	810
Phe Glu Ile Ser Arg Arg Leu Ile Asp Arg Thr Asn Ala Asn Phe Leu	
25 30 35	
GTG TGG CCG CCC TGT GTG GAG GTG CAG CGC TGC TCC GGC TGC TGC AAC	858
Val Trp Pro Pro Cys Val Glu Val Gln Arg Cys Ser Gly Cys Cys Asn	
40 45 50	
AAC CGC AAC GTG CAG TGC CGC CCC ACC CAG GTG CAG CTG CGA CCT GTC	906
Asn Arg Asn Val Gln Cys Arg Pro Thr Gln Val Gln Leu Arg Pro Val	
55 60 65 70	
CAG GTG AGA AAG ATC GAG ATT GTG CGG AAG AAG CCA ATC TTT AAG AAG	954
Gln Val Arg Lys Ile Glu Ile Val Arg Lys Lys Pro Ile Phe Lys Lys	
75 80 85	
GCC ACG GTG ACG CTG GAA GAC CAC CTG GCA TGC AAG TGT GAG ACA GTG	1002
Ala Thr Val Thr Leu Glu Asp His Leu Ala Cys Lys Cys Glu Thr Val	
90 95 100	
GCA GCT GCA CGG [*] CCT GTG ACC CGA AGC CCG GGG GGT TCC CAG GAG CAG	1050
Ala Ala Ala Arg Pro Val Thr Arg Ser Pro Gly Gly Ser Gln Glu Gln	
105 110 115	
CGA GCC AAA ACG CCC CAA ACT CGG GTG ACC ATT CGG ACG GTG CGA GTC	1098
Arg Ala Lys Thr Pro Gln Thr Arg Val Thr Ile Arg Thr Val Arg Val	
120 125 130	
CGC CGG CCC CCC AAG GGC AAG CAC CGG AAA TTC AAG CAC ACG CAT GAC	1146
Arg Arg Pro Pro Lys Gly Lys His Arg Lys Phe Lys His Thr His Asp	
135 140 145 150	
AAG ACG GCA CTG AAG GAG ACC CTT GGA GCC TAG GGGCATCGGC AGGAGAGTGT	1199
Lys Thr Ala Leu Lys Glu Thr Leu Gly Ala *	
155 160	
GTGGGCAGGG TTATTTAATA TGGTATTTGT GTATTGCCCC CATGGGGCCT TGGAGTAGAT	1259
AATATTGTTT CCCTCGTCCG TCTGTCTCGA TGCCTGATTC GGACGGCCAA TGGTGCCTCC	1319

55

C

1320

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 242 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```

Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg
-81 -80                      -75                      -70

Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met
-65                      -60                      -55                      -50

Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu
                      -45                      -40                      -35

His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met
                      -30                      -25                      -20

Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg
-15                      -10                      -5

Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu
  1                      5                      10                      15

Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp
                      20                      25                      30

Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln
                      35                      40                      45

Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr
                      50                      55                      60

Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg
  65                      70                      75

Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu
  80                      85                      90                      95

Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr Arg Ser
                      100                      105                      110

Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val
                      115                      120                      125

Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg
  130                      135                      140

Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly

```

145 150 155
Ala *
160

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Synthetic oligonucleotide (primer) "
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Synthetic (derived from Homo sapiens sequence)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CATTCCCGAG GAGCTCTACG AGATGCTGAG TGAC

34

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Synthetic oligonucleotide (primer) "
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Synthetic (derived from Homo sapiens sequence)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTTGGCTCGG GGGAAGAGGA GCCTGGG

27

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 89 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens/Saccharomyces cerevisiae derived sequence

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 44..89
(D) OTHER INFORMATION: /function= "truncated alpha factor leader/lys-arg proc./N-term. propept"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCGAGCGCAT TAGCTGCTCC AGTCAACACT ACAACAGAAG ATGAAACGGC ACAAATTCCG	60
GCTAAAGAG ACCCCATTCC CGAGGAGCT	89

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens derived sequence

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..39
(D) OTHER INFORMATION: /function= "C-term.alpha factor leader/lys-arg proc./N-term. propeptide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CCTCGGGAAT GGGGTCTCTT TTAGCCGGAA TTTGTGCCGT TTCATCTTCT GTTGTAGTGT	60
TGACTGGAGC AGCTAATGCG C	81

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens derived sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CAAGTGTGAG ACAGTGGCAG CTGCACGGCC TGTGACCTAA TAGCGTCG

48

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens derived sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TCGACGACGC TATTAGGTCA CAGGCCGTGC AGCTGCCACT GTCTCACACT TGCATG

56

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2023 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic chimera"

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens/Saccharomyces cerevisiae

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1115..1735

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION: 1..1114
(D) OTHER INFORMATION: /standard_name= "ADH/GAP promoter"

(ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION: 1115..1225
 (D) OTHER INFORMATION: /function= "mediates secretion of
rhPDGF-B"
/product= "alpha factor signal/truncated alpha
factor leader peptide"
/standard_name= "truncated alpha factor
signal/leader sequence"

(ix) FEATURE:
 (A) NAME/KEY: transit_peptide
 (B) LOCATION: 1226..1402
 (D) OTHER INFORMATION: /product= "PDGF-B propeptide"
/standard_name= "PDGF-B prosequence"

(ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1403..1735
 (D) OTHER INFORMATION: /product= "rhPDGF-B protein"
/standard_name= "rhPDGF-B"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGATCCTTCA ATATGCGCAC ATACGCTGTT ATGTTCAAGG TCCCTTCGTT TAAGAACGAA	60
AGCGGTCTTC CTTTGTAGGG ATGTTTCAAG TTGTTCAAAT CTATCAAATT TGCAAATCCC	120
CAGTCTGTAT CTAGCTAGAT ATACCAATGG CAAACTGAGC ACAACAATAC CAGTCCGGAT	180
CAACTGGCAC CATCTCTCCC GTAGTCTCAT CTAATTTTTT TCCGGATGA GGTTCCAGAT	240
ATACCGCAAC ACCTTTATTA TGGTTTCCCT GAGGGAATAA TAGAATGTCC CATTGCAAAT	300
CACCAATTCT AAACCTGGGC GAATTGTATT TCGGGTTTGT TAACTCGTTC CAGTCAGGAA	360
TGTTCCACGT GAAGCTATCT TCCAGCAAAG TCTCCACTTC TTCATCAAAT TGTGGGAGAA	420
TACTCCCAAT GCTCTTATCT ATGGGACTTC CGGGAAACAC AGTACCGATA CTCCCAATT	480
CGTCTTCAGA GCTCATTGTT TGTTTGAAGA GACTAATCAA AGAATCGTTT TCTCAAAAAA	540
ATTAATATCT TAACTGATAG TTTGATCAA GGGGCAAAAC GTAGGGGCAA ACAAACGGAA	600
AAATCGTTTC TCAAATTTTC TGATGCCAAG AACTCTAACC AGTCTTATCT AAAAATTGCC	660
TTATGATCCG TCTCTCCGGT TACAGCCTGT GTAAC TGATT AATCCTGCCT TTCTAATCAC	720
CATTCTAATG TTTTAATTAA GGGATTTTGT CTTCATTAAC GGCTTTCGCT CATAAAATG	780
TTATGACGTT TTGCCCCGAG GCGGGAAACC ATCCACTTCA CGAGACTGAT CTCCTCTGCC	840
GGAACACCGG GCATCTCCAA CTTATAAGTT GGAGAAATAA GAGAATTTC GATTGAGAGA	900
ATGAAAAAAA AAAACCCTGA AAAAAAGGT TGAAACCAGT TCCCTGAAAT TATTCCCCTA	960

CTTGACTAAT AAGTATATAA AGACGGTAGG TATTGATTGT AATTCTGTAA ATCTATTTCT	1020
TAAACTTCTT AAATTCTACT TTTATAGTTA GTCTTTTTTTT TAGTTTTTAAA ACACCAAGAA	1080
CTTAGTTTCG AATAAACACA CATAAACAAA CACC ATG AGA TTT CCT TCA ATT	1132
Met Arg Phe Pro Ser Ile	
-96 -95	
TTT ACT GCA GTT TTA TTC GCA GCC TCG AGC GCA TTA GCT GCT CCA GTC	1180
Phe Thr Ala Val Leu Phe Ala Ala Ser Ser Ala Leu Ala Ala Pro Val	
-90 -85 -80 -75	
AAC ACT ACA ACA GAA GAT GAA ACG GCA CAA ATT CCG GCT AAA AGA GAC	1228
Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln Ile Pro Ala Lys Arg Asp	
-70 -65 -60	
CCC ATT CCC GAG GAG CTC TAC GAG ATG CTG AGT GAC CAC TCG ATC CGC	1276
Pro Ile Pro Glu Glu Leu Tyr Glu Met Leu Ser Asp His Ser Ile Arg	
-55 -50 -45	
TCC TTT GAT GAT CTC CAA CGC CTG CTG CAC GGA GAC CCC GGA GAG GAA	1324
Ser Phe Asp Asp Leu Gln Arg Leu Leu His Gly Asp Pro Gly Glu Glu	
-40 -35 -30	
GAT GGG GCC GAG TTG GAC CTG AAC ATG ACC CGC TCC CAC TCT GGA GGC	1372
Asp Gly Ala Glu Leu Asp Leu Asn Met Thr Arg Ser His Ser Gly Gly	
-25 -20 -15	
GAG CTG GAG AGC TTG GCT CGG GGG AAG AGG AGC CTG GGT TCC CTG ACC	1420
Glu Leu Glu Ser Leu Ala Arg Gly Lys Arg Ser Leu Gly Ser Leu Thr	
-10 -5 1 5	
ATT GCT GAG CCG GCC ATG ATC GCC GAG TGC AAG ACG CGC ACC GAG GTG	1468
Ile Ala Glu Pro Ala Met Ile Ala Glu Cys Lys Thr Arg Thr Glu Val	
10 15 20	
TTC GAG ATC TCC CGG CGC CTC ATA GAC CGC ACC AAC GCC AAC TTC CTG	1516
Phe Glu Ile Ser Arg Arg Leu Ile Asp Arg Thr Asn Ala Asn Phe Leu	
25 30 35	
GTG TGG CCG CCC TGT GTG GAG GTG CAG CGC TGC TCC GGC TGC TGC AAC	1564
Val Trp Pro Pro Cys Val Glu Val Gln Arg Cys Ser Gly Cys Cys Asn	
40 45 50	
AAC CGC AAC GTG CAG TGC CGC CCC ACC CAG GTG CAG CTG CGA CCT GTC	1612
Asn Arg Asn Val Gln Cys Arg Pro Thr Gln Val Gln Leu Arg Pro Val	
55 60 65 70	
CAG GTG AGA AAG ATC GAG ATT GTG CGG AAG AAG CCA ATC TTT AAG AAG	1660
Gln Val Arg Lys Ile Glu Ile Val Arg Lys Lys Pro Ile Phe Lys Lys	
75 80 85	
GCC ACG GTG ACG CTG GAA GAC CAC CTG GCA TGC AAG TGT GAG ACA GTG	1708
Ala Thr Val Thr Leu Glu Asp His Leu Ala Cys Lys Cys Glu Thr Val	
90 95 100	
GCA GCT GCA CGG CCT GTG ACC TAA TAG CGTCGTCGAC TTTGTTCCCA	1755

Ala Ala Ala Arg Pro Val Thr * *
 105 110

CTGTACTTTT AGCTCGTACA AAATACAATA TACTTTTCAT TTCTCCGTAA ACAACATGTT 1815
 TTCCCATGTA ATATCCTTTT CTATTTTTCG TTCCGTTACC AACTTTACAC ATACTTTATA 1875
 TAGCTATTCA CTTCTATACA CTAAAAAACT AAGACAATTT TAATTTTGCT GCCTGCCATA 1935
 TTTCAATTTG TTATAAATTC CTATAATTTA TCCTATTAGT AGCTAAAAAA AGATGAATGT 1995
 GAATCGAATC CTAAGAGAAT TCGGATCC 2023

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 207 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
 -96 -95 -90 -85

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
 -80 -75 -70 -65

Ile Pro Ala Lys Arg Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met Leu
 -60 -55 -50

Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu His
 -45 -40 -35

Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met Thr
 -30 -25 -20

Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Lys Arg
 -15 -10 -5

Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu Cys
 1 5 10 15

Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp Arg
 20 25 30

Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln Arg
 35 40 45

Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr Gln
 50 55 60

Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg Lys
 65 70 75 80

```

Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu Ala
      85                      90                      95
Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr * *
      100                      105                      110

```

(2) INFORMATION FOR SEQ ID NO:38:

```

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 480 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
    (A) DESCRIPTION: /desc = "Chimeric DNA molecule"

(vi) ORIGINAL SOURCE:
    (A) ORGANISM: Homo sapiens/Saccharomyces cerevisiae

(ix) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION: 1..471

(ix) FEATURE:
    (A) NAME/KEY: misc_feature
    (B) LOCATION: 1..255
    (D) OTHER INFORMATION: /function= "mediates protein
secretion"
/product= "Yeast alpha factor leader peptide"
/standard_name= "Alpha factor signal/leader
sequence"

(ix) FEATURE:
    (A) NAME/KEY: mat_peptide
    (B) LOCATION: 256..471
    (D) OTHER INFORMATION: /product= "rhIGF-I-A protein"
/standard_name= "rhIGF-I-A"

```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```

ATG AGA TTT CCT TCA ATT TTT ACT GCA GTT TTA TTC GCA GCA TCC TCC      48
Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
-85                      -80                      -75                      -70

GCA TTA GCT GCT CCA GTC AAC ACT ACA ACA GAA GAT GAA ACG GCA CAA      96
Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
-65                      -60                      -55

ATT CCG GCT GAA GCT GTC ATC GGT TAC TTA GAT TTA GAA GGG GAT TTC      144
Ile Pro Ala Glu Ala Val Ile Gly Tyr Leu Asp Leu Glu Gly Asp Phe
-50                      -45                      -40

```


GAT GTT GCT GTT TTG CCA TTT TCC AAC AGC ACA AAT AAC GGG TTA TTG	192
Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu	
-35 -30 -25	
TTT ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG GTA	240
Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val	
-20 -15 -10	
CAG CTG GAT AAA AGA GGT CCA GAA ACC TTG TGT GGT GCT GAA TTG GTC	288
Gln Leu Asp Lys Arg Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val	
-5 1 5 10	
GAT GCT TTG CAA TTC GTT TGT GGT GAC AGA GGT TTC TAC TTC AAC AAG	336
Asp Ala Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys	
15 20 25	
CCA ACC GGT TAC GGT TCT TCT TCT AGA AGA GCT CCA CAA ACC GGT ATC	384
Pro Thr Gly Tyr Gly Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile	
30 35 40	
GTT GAC GAA TGT TGT TTC AGA TCT TGT GAC TTG AGA AGA TTG GAA ATG	432
Val Asp Glu Cys Cys Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met	
45 50 55	
TAC TGT GCT CCA TTG AAG CCA GCT AAG TCT GCT TGA TAA GTCGACTTT	480
Tyr Cys Ala Pro Leu Lys Pro Ala Lys Ser Ala * *	
60 65 70	

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 157 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser	
-85 -80 -75 -70	
Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln	
-65 -60 -55	
Ile Pro Ala Glu Ala Val Ile Gly Tyr Leu Asp Leu Glu Gly Asp Phe	
-50 -45 -40	
Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu	
-35 -30 -25	
Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val	
-20 -15 -10	
Gln Leu Asp Lys Arg Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val	
-5 1 5 10	

```

Asp Ala Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys
      15                      20                      25
Pro Thr Gly Tyr Gly Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile
      30                      35                      40
Val Asp Glu Cys Cys Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met
      45                      50                      55
Tyr Cys Ala Pro Leu Lys Pro Ala Lys Ser Ala * *
      60                      65                      70

```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 621 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "Chimeric DNA molecule"

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens/Saccharomyces cerevisiae

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..579

(ix) FEATURE:

```

(A) NAME/KEY: misc_feature
(B) LOCATION: 1..255
(D) OTHER INFORMATION: /function= "mediates secretion of
protein"
/product= "3'end of yeast alpha factor leader
peptide"
/standard_name= "alpha factor leader/signal
sequence"

```

(ix) FEATURE:

```

(A) NAME/KEY: mat_peptide
(B) LOCATION: 256..471
(D) OTHER INFORMATION: /product= "rhIGF-I-A protein"
/standard_name= "rhIGF-I-A"

```

(ix) FEATURE:

```

(A) NAME/KEY: transit_peptide
(B) LOCATION: 472..579
(D) OTHER INFORMATION: /function= "mediates protein
transport/translocation"
/product= "IGF-I-A propeptide"
/standard_name= "IGF-I-A prosequence"

```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ATG AGA TTT CCT TCA ATT TTT ACT GCA GTT TTA TTC GCA GCA TCC TCC	48
Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser	
-85 -80 -75 -70	
GCA TTA GCT GCT CCA GTC AAC ACT ACA ACA GAA GAT GAA ACG GCA CAA	96
Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln	
-65 -60 -55	
ATT CCG GCT GAA GCT GTC ATC GGT TAC TTA GAT TTA GAA GGG GAT TTC	144
Ile Pro Ala Glu Ala Val Ile Gly Tyr Leu Asp Leu Glu Gly Asp Phe	
-50 -45 -40	
GAT GTT GCT GTT TTG CCA TTT TCC AAC AGC ACA AAT AAC GGG TTA TTG	192
Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu	
-35 -30 -25	
TTT ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG GTA	240
Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val	
-20 -15 -10	
CAG CTG GAT AAA AGA GGT CCA GAA ACC TTG TGT GGT GCT GAA TTG GTC	288
Gln Leu Asp Lys Arg Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val	
-5 1 5 10	
GAT GCT TTG CAA TTC GTT TGT GGT GAC AGA GGT TTC TAC TTC AAC AAG	336
Asp Ala Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys	
15 20 25	
CCA ACC GGT TAC GGT TCT TCT TCT AGA AGA GCT CCA CAA ACC GGT ATC	384
Pro Thr Gly Tyr Gly Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile	
30 35 40	
GTT GAC GAA TGT TGT TTC AGA TCT TGT GAC TTG AGA AGA TTG GAA ATG	432
Val Asp Glu Cys Cys Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met	
45 50 55	
TAC TGT GCT CCA TTG AAG CCT GCT AAG TCT GCT AAA AGA TCC GTC AGA	480
Tyr Cys Ala Pro Leu Lys Pro Ala Lys Ser Ala Lys Arg Ser Val Arg	
60 65 70 75	
GCT CAA AGA CAC ACC GAT ATG CCA AAG ACC CAA AAG GAA GTT CAC TTG	528
Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Glu Val His Leu	
80 85 90	
AAG AAC GCT TCC AGA GGT TCT GCT GGT AAC AAG AAC TAC AGA ATG TGA	576
Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys Asn Tyr Arg Met *	
95 100 105	
TAA GTCGACTTTG TTCCCACTGT ACTTTTAGCT CGTACAAAAT AC	621

*

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 193 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
-85                -80                -75                -70

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
                -65                -60                -55

Ile Pro Ala Glu Ala Val Ile Gly Tyr Leu Asp Leu Glu Gly Asp Phe
                -50                -45                -40

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu
                -35                -30                -25

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val
                -20                -15                -10

Gln Leu Asp Lys Arg Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val
-5                1                5                10

Asp Ala Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys
                15                20                25

Pro Thr Gly Tyr Gly Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile
                30                35                40

Val Asp Glu Cys Cys Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met
                45                50                55

Tyr Cys Ala Pro Leu Lys Pro Ala Lys Ser Ala Lys Arg Ser Val Arg
60                65                70                75

Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Glu Val His Leu
                80                85                90

Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys Asn Tyr Arg Met  *
                95                100                105

```

*

5 WHAT IS CLAIMED IS:

1. A nucleotide sequence comprising in the 5' to 3' direction and operably linked
(a) a yeast-recognized transcription and translation initiation region, (b) a coding
sequence for a hybrid precursor polypeptide, and (c) a yeast-recognized transcription
and translation termination region, wherein said hybrid precursor polypeptide
comprises:

5'-SP-(PS)_{n-1}-(LP-PS)_{n-2}-(NPRO_{MHP}-PS)_{n-3}-MHP-(PS-CPRO_{MHP})_{n-4}-3'

wherein:

SP comprises a signal peptide sequence for a yeast secreted protein;

PS comprises a preferred processing site cleaved in vivo by a yeast proteolytic
enzyme;

LP comprises a leader peptide sequence for a yeast secreted protein;

NPRO_{MHP} comprises a native N-terminal propeptide sequence of a mature
heterologous mammalian protein of interest;

MHP comprises a peptide sequence for said mature heterologous mammalian
protein of interest;

CPRO_{MHP} comprises a native C-terminal propeptide sequence of said mature
heterologous mammalian protein of interest; and

n-1, n-2, n-3, and n-4 independently = 0 or 1;

wherein said processing sites allow for proteolytic processing of said precursor
polypeptide to said mature protein in vivo by a yeast host cell, and wherein at
least n-3 or n-4 = 1.

2. The nucleotide sequence of claim 1, wherein said mammalian protein is a
PDGF protein or an IGF protein, or variants thereof.

3. The nucleotide sequence of claim 2, wherein said protein is a human protein.

4. The nucleotide sequence of claim 3, wherein said human PDGF is PDGF-BB
or variants thereof.

- 5 5. The nucleotide sequence of claim 4, wherein SP is a signal peptide sequence for a *Saccharomyces cerevisiae* α -factor.
6. The nucleotide sequence of claim 5, wherein said α -factor is *Mat α* or variants thereof.
- 10 7. The nucleotide sequence of claim 6, wherein $n-2 = 1$, $n-3 = 1$, and $n-4 = 0$.
8. The nucleotide sequence of claim 7, wherein LP is a truncated leader peptide sequence.
- 15 9. The nucleotide sequence of claim 8, wherein said coding sequence for the hybrid precursor polypeptide has the nucleotide sequence set forth in SEQ ID NO. 26.
- 20 10. The nucleotide sequence of claim 8, wherein said hybrid precursor polypeptide has the amino acid sequence set forth in SEQ ID NO. 27.
11. The nucleotide sequence of claim 3, wherein $n-3 = 0$ and $n-4 = 1$ and said human IGF protein is IGF-I-A or variants thereof.
- 25 12. The nucleotide sequence of claim 11, wherein SP is a signal peptide sequence for a *Saccharomyces cerevisiae* α -factor.
13. The nucleotide sequence of claim 12, wherein said α -factor is *Mat α* or variants thereof.
- 30 14. The nucleotide sequence of claim 13, wherein said coding sequence for said hybrid precursor polypeptide has the nucleotide sequence set forth in SEQ ID NO. 40.
- 35 15. The nucleotide sequence of claim 13, wherein said hybrid precursor polypeptide has the amino acid sequence set forth in SEQ ID NO. 41.

16. A vector comprising a nucleotide sequence that comprises in the 5' to 3' direction and operably linked (a) a yeast-recognized transcription and translation initiation region, (b) a coding sequence for a hybrid precursor polypeptide, and (c) a yeast-recognized transcription and translation termination region, wherein said hybrid precursor polypeptide comprises:



wherein:

SP comprises a signal peptide sequence for a yeast secreted protein;

PS comprises a preferred processing site cleaved in vivo by a yeast proteolytic enzyme;

LP comprises a leader peptide sequence for a yeast secreted protein;

NPRO_{MHP} comprises a native N-terminal propeptide sequence of a mature heterologous mammalian protein of interest;

MHP comprises a peptide sequence for said mature heterologous mammalian protein of interest;

CPRO_{MHP} comprises a native C-terminal propeptide sequence of said mature heterologous mammalian protein of interest; and

n-1, n-2, n-3, and n-4 independently = 0 or 1;

wherein said processing sites allow for proteolytic processing of said precursor polypeptide to said mature protein in vivo by a yeast host cell, and wherein at least n-3 or n-4 = 1.

17. The vector of claim 16, wherein said vector is the yeast shuttle vector pAB24.

18. A yeast host cell stably transformed with a nucleotide sequence comprising an expression cassette, said cassette comprising in the 5' to 3' direction and operably linked (a) a yeast-recognized transcription and translation initiation region, (b) a coding sequence for a hybrid precursor polypeptide, and (c) a yeast-recognized transcription and translation termination region, wherein said hybrid precursor polypeptide comprises:



5

wherein:

SP comprises a signal peptide sequence for a yeast secreted protein;

PS comprises a preferred processing site cleaved in vivo by a yeast proteolytic enzyme;

10

LP comprises a leader peptide sequence for a yeast secreted protein;

NPRO_{MHP} comprises a native N-terminal propeptide sequence of a mature heterologous mammalian protein of interest;

MHP comprises a peptide sequence for said mature heterologous mammalian protein of interest;

15

CPRO_{MHP} comprises a native C-terminal propeptide sequence of said mature heterologous mammalian protein of interest; and

n-1, n-2, n-3, and n-4 independently = 0 or 1;

wherein said processing sites allow for proteolytic processing of said precursor polypeptide to said mature protein in vivo by a yeast host cell, and wherein at

20

least n-3 or n-4 = 1.

19. The cell of claim 18, wherein said processing sites are dipeptides cleaved by the KEX2 gene product of *Saccharomyces*.

25

20. The cell of claim 19, wherein said dipeptides are 5'-Lys-Arg-3'.

21. The cell of claim 20, wherein said yeast cell is from the genus *Saccharomyces*.

30

22. The cell of claim 21, wherein said yeast cell is *S. cerevisiae*.

23. A method for expression of heterologous proteins and their secretion in the biologically active mature form using a yeast host cell as the expression system, said method comprising transforming said yeast cell with a vector comprising a nucleotide sequence that comprises in the 5' to 3' direction and operably linked (a) a yeast-recognized transcription and translation initiation region, (b) a coding sequence for a hybrid precursor polypeptide, and (c) a yeast-recognized transcription and translation termination region, wherein said hybrid precursor polypeptide comprises:

35

5 5'-SP-(PS)_{n-1}-(LP-PS)_{n-2}-(NPRO_{MHP}-PS)_{n-3}-MHP-(PS-CPRO_{MHP})_{n-4}-3'

wherein:

SP comprises a signal peptide sequence for a yeast secreted protein;

10 PS comprises a preferred processing site cleaved in vivo by a yeast proteolytic
 enzyme;

LP comprises a leader peptide sequence for a yeast secreted protein;

NPRO_{MHP} comprises a native N-terminal propeptide sequence of a mature
 heterologous mammalian protein of interest;

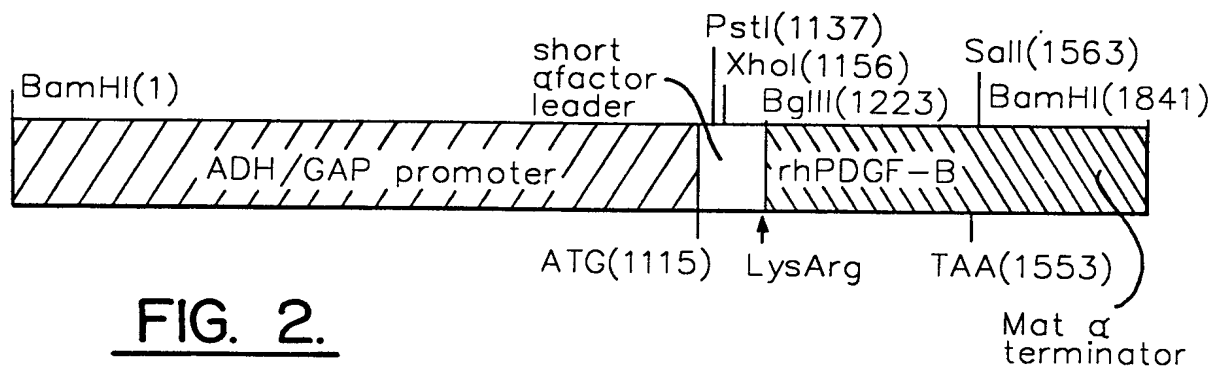
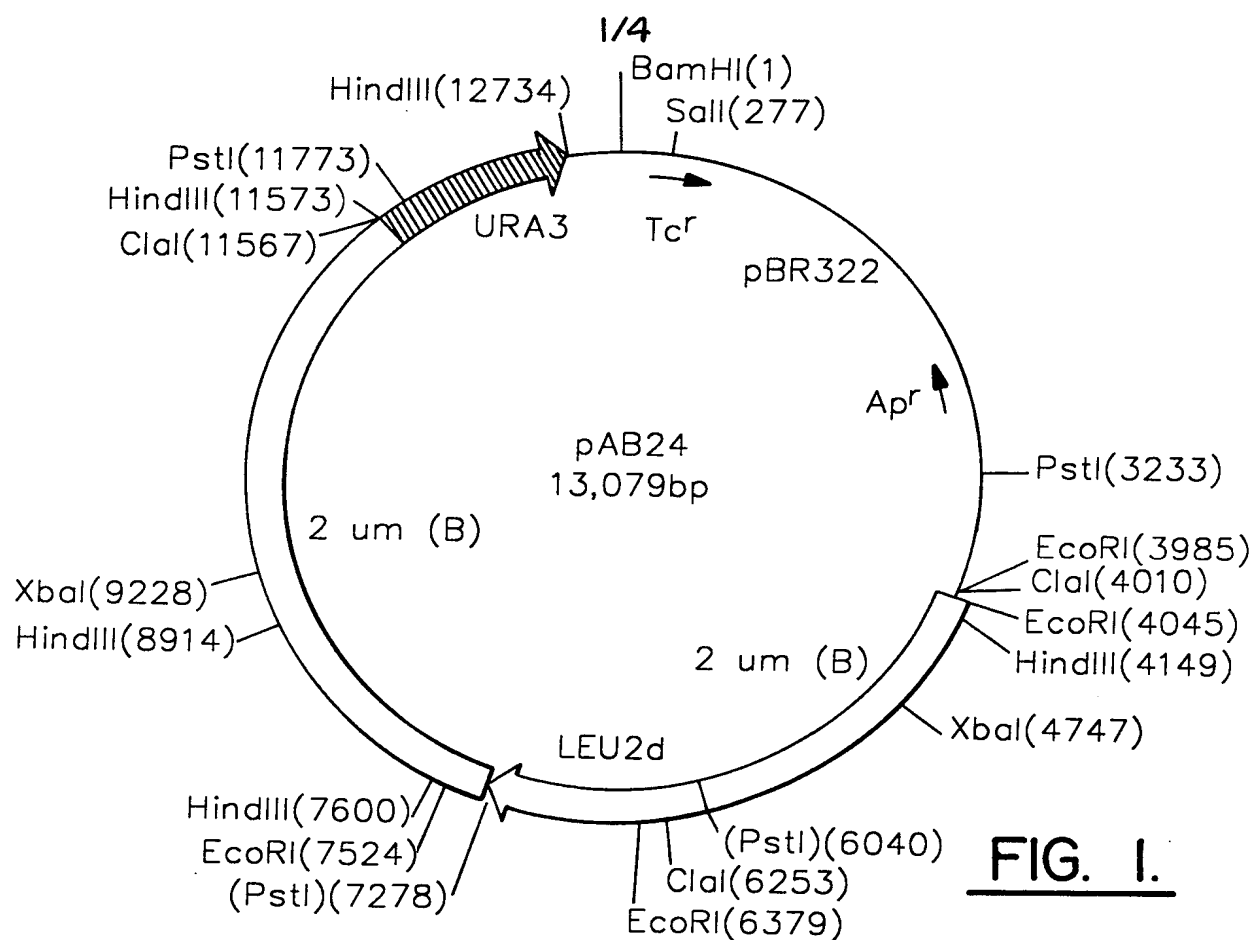
15 MHP comprises a peptide sequence for said mature heterologous mammalian
 protein of interest;

CPRO_{MHP} comprises a native C-terminal propeptide sequence of said mature
 heterologous mammalian protein of interest; and

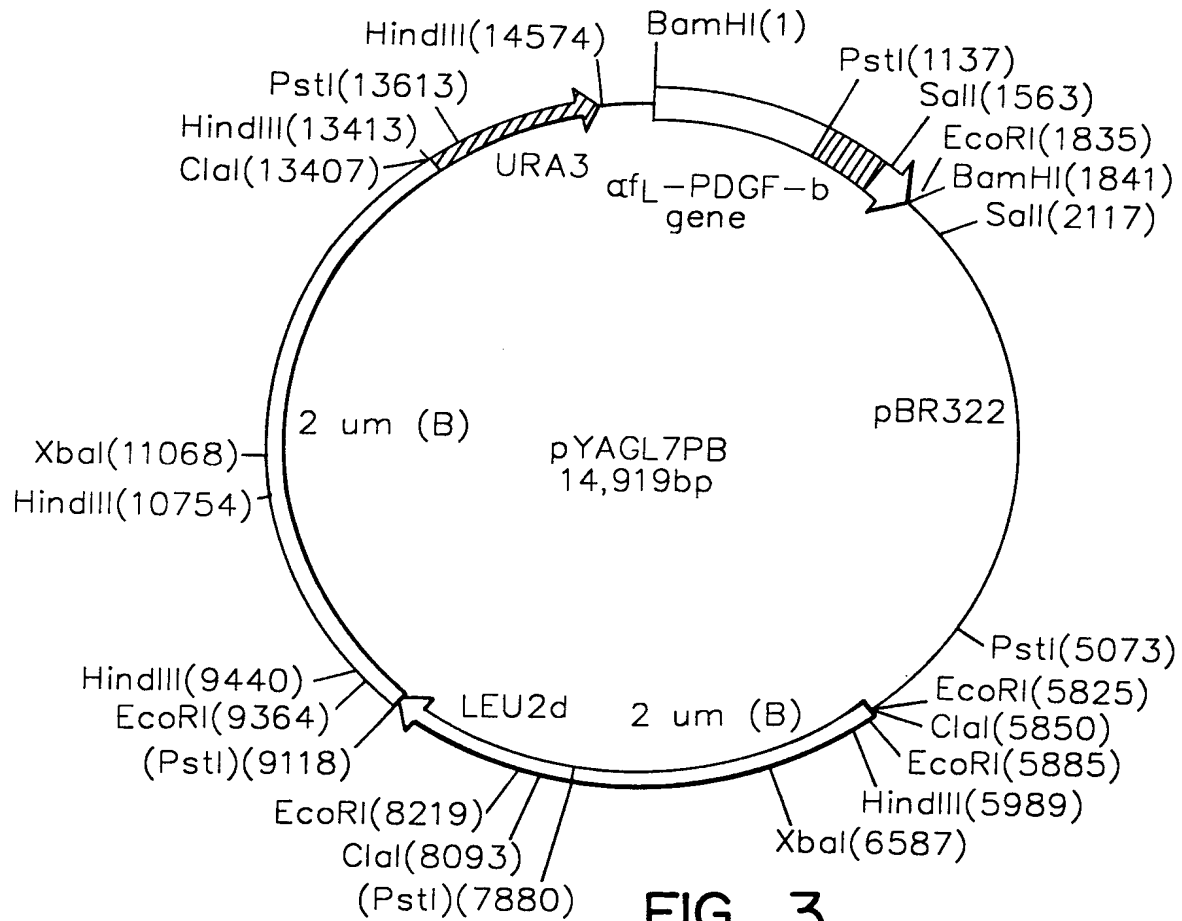
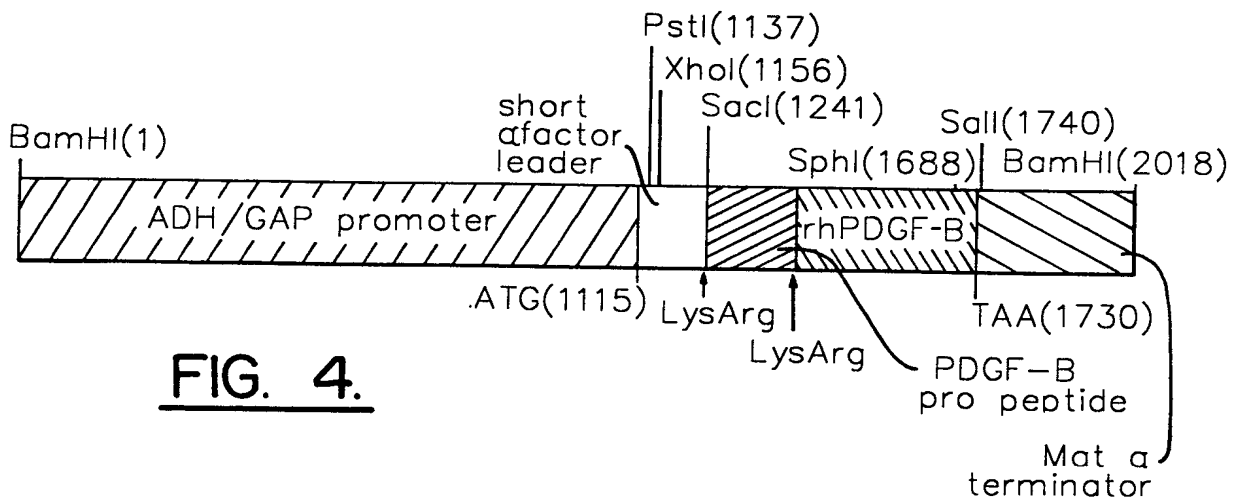
n-1, n-2, n-3, and n-4 independently = 0 or 1;

20 wherein said processing sites allow for proteolytic processing of said precursor
 polypeptide to said mature protein in vivo by a yeast host cell, and wherein at
 least n-3 or n-4 = 1.

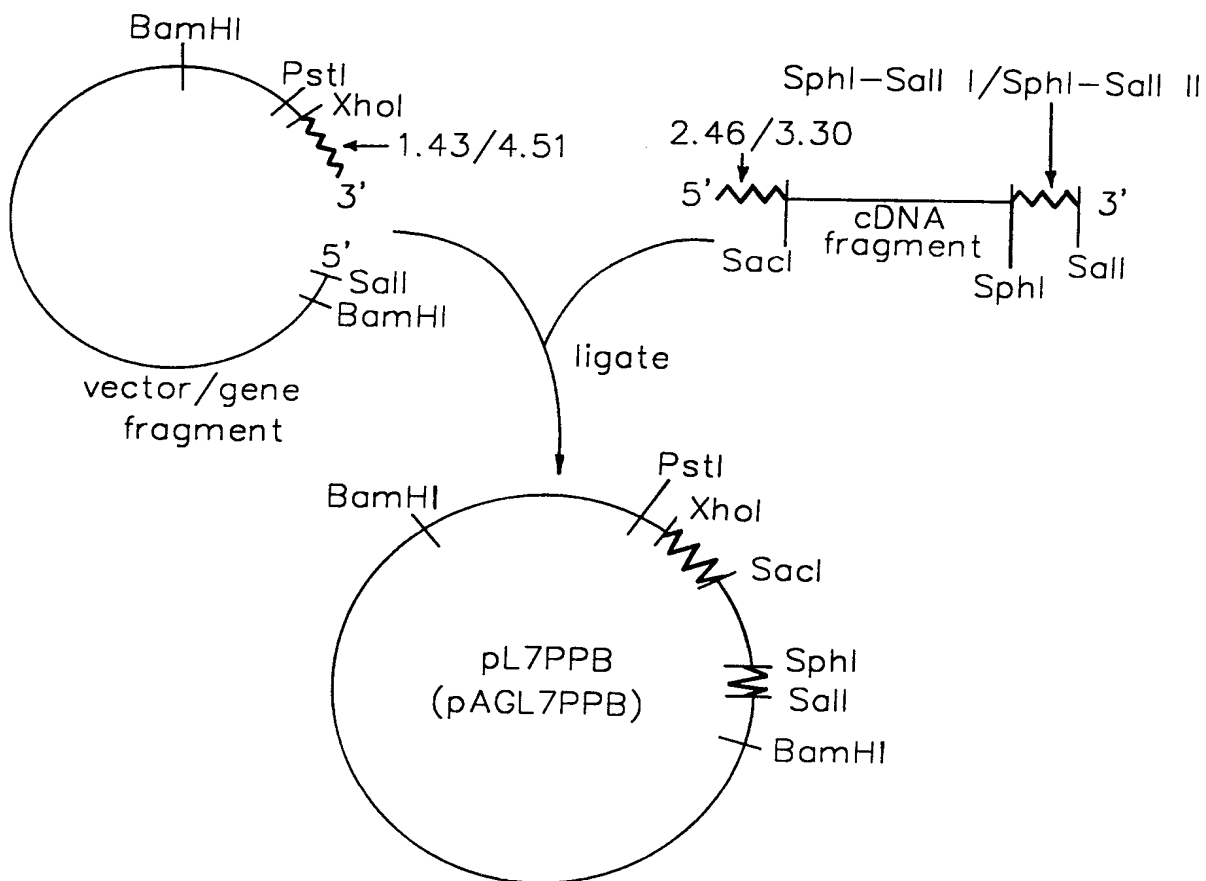
25



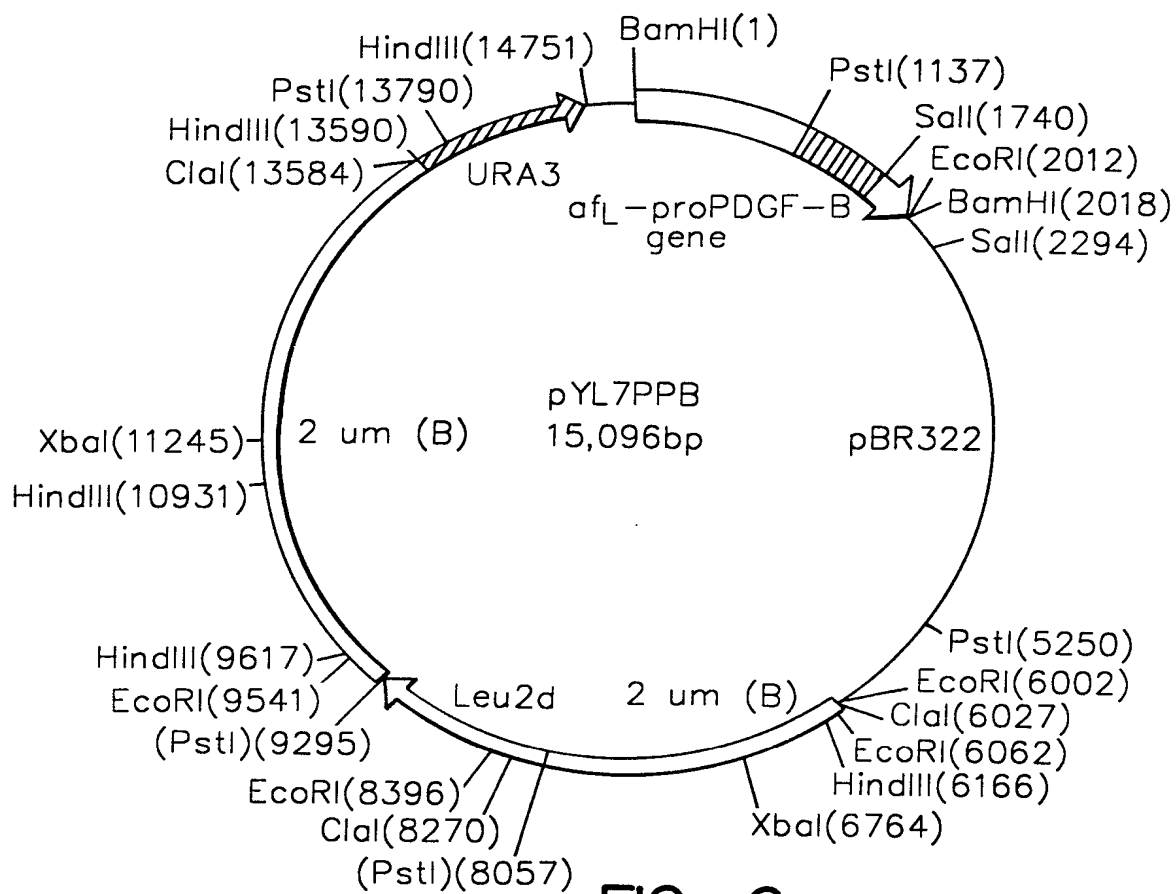
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**FIG. 3.****FIG. 4.**

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FIG. 5.

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**FIG. 6.**

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/22647

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/81 C07K14/49 C07K14/65 C12N1/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 02059 A (NOVONORDISK AS) 19 January 1995 see the whole document ---	1-23
Y	WO 95 35384 A (NOVONORDISK AS) 28 December 1995 see the whole document ---	1-23
Y	EP 0 324 274 A (CHIRON CORP.) 19 July 1989 cited in the application see the whole document ---	1-23
Y	US 5 187 263 A (MURRAY MARK J. ET AL.) 16 February 1993 cited in the application see the whole document ---	1-23
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

6 May 1998

Date of mailing of the international search report

25/05/1998

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INTERNATIONAL SEARCH REPORT

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